FIGURE 1A

FIGURE 1 B

PART ONE: TRIGGER REACTION
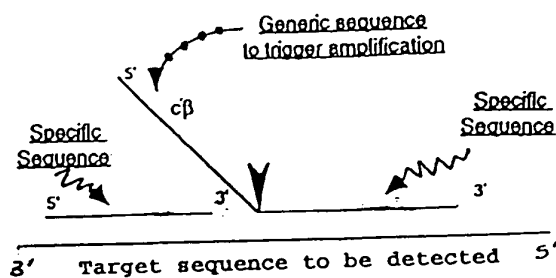
PART TWO: DETECTION REACTION

Denature, anneal

Denature, anneal

4 cuts

FIGURE 2

```
MAJORITY  (SEQ ID NO:7)   ATGXXGGCGATGCTTCGCCTCTTTGAGCCCAAAGGCCCGGTCCTCCTGGTGGACGGGCCACCACCTGGCCT   70

DNAPTAO   (SEQ ID NO:1)   ....AG..G.......................G...................................... 67
DNAPTRL   (SEQ ID NO:2)   ........G......A...............C.G..................................... 70
DNAPTTH   (SEQ ID NO:3)   ....GA.............G..........................GCCGCGGGTCTACGGCTT        70

MAJORITY                  ACCGCACCTTCTTCGCCCTGAAGGGCCTCACCACCAGCCGGGGCCGGGGCGAACCGGTGCAGGCGGGTCTACGGCTT   140

DNAPTAO                   .......CA....................G..G.................................... 137
DNAPTRL                   ......T....C................C......C..T............................. 140
DNAPTTH                   ..............................G...................................... 140

MAJORITY                  CGCCAAGAGCCTCCTCAAGGCCCTGAAGGAGGGGACXXGGCGGTGXTCGTGGTCTTTGACGCCCAAG   207

DNAPTAO                   ........C........................A.................................. 204
DNAPTRL                   ........A...........GT..T.......................................... 210
DNAPTTH                   .................T..AA...C..CT.....................................

MAJORITY                  GCCCCTCCTTCGCGCCACGGAGGCCTACAAGGCGGGCGGGCCCCCACCCCCGGAGGACTTTC   277

DNAPTAO                   ................G..GG..............................G.............. 274
DNAPTRL                   .......................GA......G.......C.........C............... 280
DNAPTTH                   ...................................................................

MAJORITY                  CCCGGCAGCTCGCCCCTCATCAAGGAGCTGGTGGACCTCCTGGGCGCCTTGCGCGGCCTTCGAGGTCCCCGGGCTA   347

DNAPTAO                   .........A.............T...........................G..........G....... 344
DNAPTRL                   ..........G...................A...C...T...G..G....T...               350
DNAPTTH                   .......................T.A.C.........................................
```
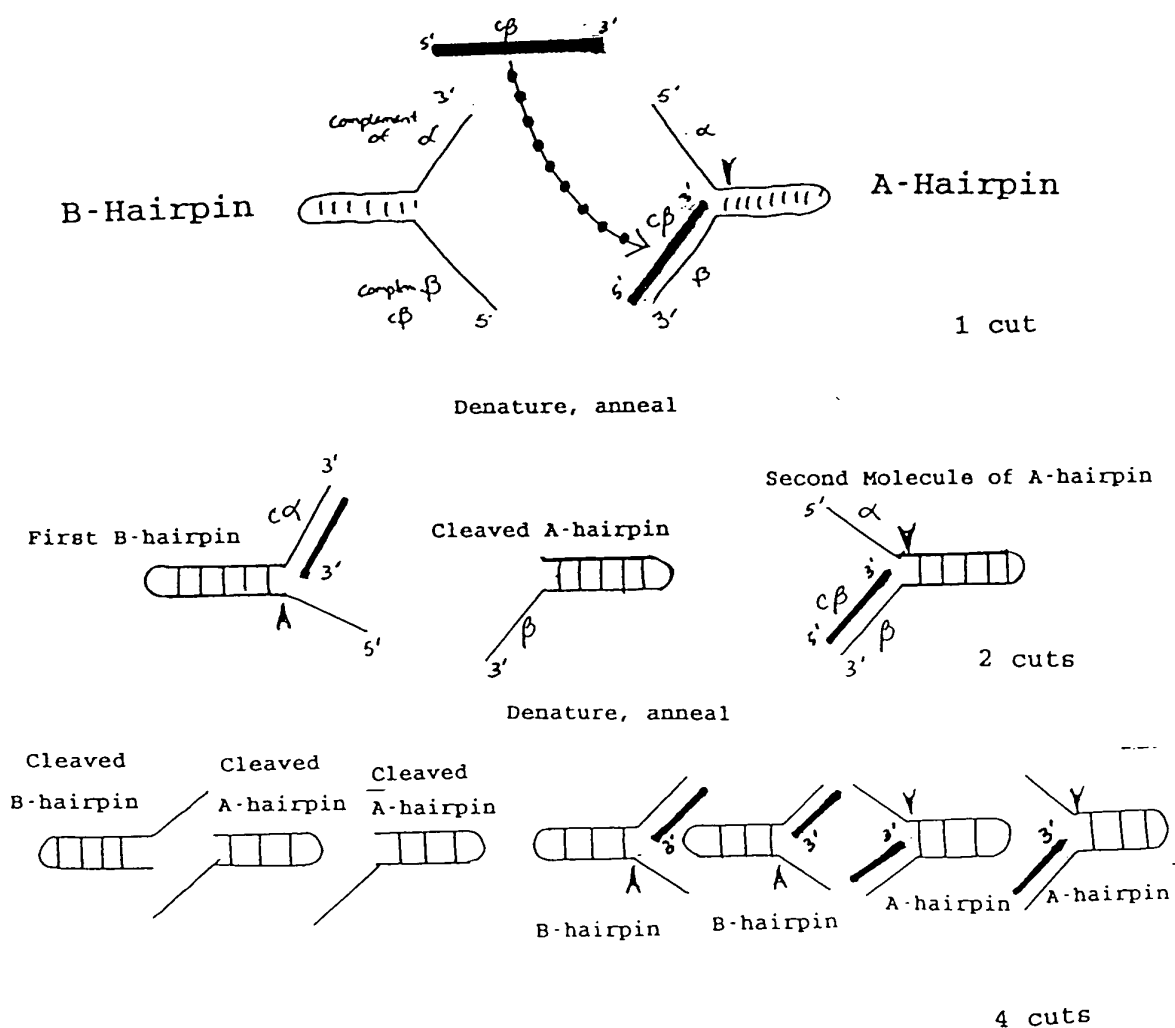
FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)   CGAGCGCGGACGACGTXCTGGCCACCCTGGCCAAGAGCGGGAAAAGGAGGGGTACGAGGTGCGCATCCTC

DNAPTAQ   (SEQ ID NO:1)   ...................................C..............C.............  417
DNAPTFL   (SEQ ID NO:2)   ...............G.......................CG.....................  414
DNAPTTH   (SEQ ID NO:3)   .....T.C.....................................................  420

MAJORITY                  ACCCCCGACCCGGACCTCTACCAGCTCCTTTCCGACCCGCATCGCCGTCCTCCACCCGGAGGGGTACCTCA

DNAPTAQ                   .........AAA.....T.................................CA..........  487
DNAPTFL                   ...T................G.G.....A....T...........G.          484
DNAPTTH                   ...............A.G.C......G................CC......          490

MAJORITY                  TCACCCCGGCGTGGCTTTGGGAGAAGTACGGGCCTGAGCCCGAGCCACGTGGGTGGACTACCCGGCCCCTGGC

DNAPTAQ                   .C........A.................C.C....CC.........A.            557
DNAPTFL                   .....AC.........C.C...............            554
DNAPTTH                   ...A.................C..............T.C.....C.T            560

MAJORITY                  GGGGGACCCCTCCGACAACCTCCCCGCGGGTCAAGGGCATCGGGGAGAAGACCCCCXGAAGCTCCTCXAG

DNAPTAQ                   C.....CAG...............T......G.GAG.....T..GG...          627
DNAPTFL                   .....G.T..A......G.............A.G...A..CGC..          624
DNAPTTH                   ...........A......................TC..........A..          630

MAJORITY                  CAGTGGGGGAGCCTGGAAAACCTCCTCAAGAACCTGGACCGGGTGAAGCCCGC---CXTCGGGGAGAAGA

DNAPTAQ                   ...............GC..................C..........A.          694
DNAPTFL                   .....T.C.C..........T.G...........T.G......C       691
DNAPTTH                   .......A...........A....A..A.AAAA.G......         700
```

FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)   TCCAGGCCCACATGGAXGACCTGAXGCTCTCCTGGGAGCTXTCCCAGGTGGCCACCGACCTGCCCCTGGA

DNAPTAQ (SEQ ID NO:1)   ........T.......C..T.....A......C.GG..A.........................   761
DNAPTFL (SEQ ID NO:2)   .........GGG....G.C....GCC.T...C.A...T...........C.............   764/770
DNAPTTH (SEQ ID NO:3)   ...A.......C...A.....C.G......T.........G......C............C....

MAJORITY   GGTGGACTTCGCCCAAGXGCGGGGGAGCCCGACCGGGAGGGGCTTAGGGCCTTTCTGGAGAGGCTGGAGTTT

DNAPTAQ   ...........AA.........A.................T..............................   834
DNAPTFL   ..........GG.G.C.C..CACA...A..T......GC...T....T....C..T...........   831
DNAPTTH   ...........C...C..G.......C............T.........C.................C.....   840

MAJORITY   GGCAGCCTCCTCCACCGAGTTCGGCCTCGGACGGCCCTCCTGGAGGCCCAAGGCCCTGGAGGAGCCCCCCCTGCGCCCCCCC

DNAPTAQ   .............T.....AA.................T.........................   904
DNAPTFL   ......A.........G.......G..G...G..GGCA..............T....   901
DNAPTTH   ..................C...........GCCC....C...........   910

MAJORITY   CGGAAGGGGCCCTTCGTGGGCTTTGTCCTTCCCGCCCCCAGCCCATGTGGGCGGAGCTTCTGGGCCCTGCC

DNAPTAQ   ............G.........AAG..........T...................................   974
DNAPTFL   ............T..TT.......TC.T.......T..........G.....AAA............   971
DNAPTTH   ..............C.......C..............................   980

MAJORITY   CGCCCCCAGGGAGGGGCGGGGGTCCACCGGGCCACCAGACCCCTTAXGGGCCTXAGGGGACCTXAAGGAGGTG

DNAPTAQ   ........G....C.C..G..T.A..AA.C...C.....G.....G.....C.   1044
DNAPTFL   .T.GG..GT....G..CC......A...G...........G......T...G.....   1041
DNAPTTH   .....TG....C..........G...........GGC...G..A.A...C.........C.....   1050

MAJORITY (SEQ ID NO:7)
DNAPTAQ (SEQ ID NO:1)
DNAPTFL (SEQ ID NO:2)
DNAPTTH (SEQ ID NO:3)

FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)   CGGGGXCTCCTCGCCCAAGGACCTGGCCGTTTGGCCCTGAGGGAGGGCCTXGACCTCXTGCCCGGGGACG

DNAPTAQ (SEQ ID NO:1)   .....G..T.....A......AG....C..........A....T.G....CC.....C..... 1114
DNAPTFL (SEQ ID NO:2)   .....AA...G..........C..............G......T.C..A.A.... 1111
DNAPTTH (SEQ ID NO:3)   .....C...........C.......TC.......G.A......G...... 1120

MAJORITY   ACCCCATGCTCCTCGCCTACCTCCTGGACCCCTCCAACACCACCCCCGAGGGGTGCCCCGGGCCCTACGG

DNAPTAQ   ....T........................ 1184
DNAPTFL   .............G......T..........T...... 1181
DNAPTTH   ....G................ 1190

MAJORITY   CGGGGAGTGGACGGAGGAXGCGGGGGAGCGGGGCCCTCCTXTCCGAGAGGCTCTTCCXGAACCTXXXGGAG

DNAPTAQ   C............G.......GC...T.........GCC....GTG...G. 1254
DNAPTFL   .........T...A......GG....A..C....A..C....AAA.... 1251
DNAPTTH   ......C..C.CCC.C.....C..G.......CAT.G.....CCTTA.. 1260

MAJORITY   CGCCTTGAGGGGGGAGGAGAGGCTCCTTGGCTTTACCAGGAGGTGGAGAAGCCCCTTTCCCGGGTCCTGG

DNAPTAQ   A.G.......A..A..A..AC..C..G.......G.........GCT.... 1324
DNAPTFL   .............C.................G......GT... 1321
DNAPTTH   ..........A.........C........C...... 1330

MAJORITY   CCCACATGGAGGGCCACGGGGGTXCGGCTGGACCTGGGCCTACCTCCAGGCCCTXCCCTGGAGGTGGGGA

DNAPTAQ   ....G.C...........G.C.........T...AG...T.G...........C. 1394
DNAPTFL   ....GG..................C..............A..C... 1391
DNAPTTH   .........A.........T...........C.T... 1400

FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  GGAGATCCCGCCCCCTCGGAGGAGGAGGTCTTCCCCCTGCCCGGGCCACCCCTTCAACCTCAACTCCCGGGAC

DNAPTAQ   (SEQ ID NO:1)  ......GC..............GC.....................................          1464
DNAPTFL   (SEQ ID NO:2)  ....G.G....AG..G.................T....G.......C...                     1461
DNAPTTH   (SEQ ID NO:3)  .....................................................................  1470

MAJORITY                 CAGCTCGGAAAGGGTGCTCTTTGACGAGCTXGGGCTTCCCGGCCATCGGCAAGACCGGAGAGACXGGCAAGC

DNAPTAQ                  ...........C..................A...........C.........................   1534
DNAPTFL                  .........GC.........G.C..G.T.....G.C..T..G.......A..........          1531
DNAPTTH                  .....................TA........T.G.G.....C.A.......                    1540

MAJORITY                 GCTCCACCAGCGCCCCGCCCGTGCTGAGGCCCTXCGXGAGGCCCATCGTCGGAGAAGATCCTGCAGTA

DNAPTAQ                  ...............C..............C.C.....C.C..........                    1604
DNAPTFL                  ...............T.........G.A......G.A............C.C.                  1601
DNAPTTH                  ...................G..........A.G.......C...C.                         1610

MAJORITY                 CCGGGAGCTCACCAAGCTCAAGAACACCTACATXGACCCCCTGCCXGXCCTGCTCCACCCCAGGACGGGC

DNAPTAQ                  ...............G...G...........T...G.A...A....                        1674
DNAPTFL                  .........................A......C.C...G...A...C.                      1671
DNAPTTH                  ..........................G.G.....C.AAG......G......                  1680

MAJORITY                 CGCCTCCACACCCGGCTTCAACCAGACGGGCCAGGCTTAGTAGCTCCGACCCCAACCTGC

DNAPTAQ                  ..........................A...........T.........C.                    1744
DNAPTFL                  .G..................C....TCC..........                                1741
DNAPTTH                  ............G.......G.......                                          1750
```

FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  AGAACATCCCCGTCCCGCACCCCXCTGGGCCAGAGGATCCGCGCCGGGCCTTCGTGGCCGAGGAGGGXTGGGT

DNAPTAQ   (SEQ ID NO:1)  ...............G.T.G.........................A.C...........G...C..  1814
DNAPTFL   (SEQ ID NO:2)  .............G..........C.C..........A...............C.......C.....  1811
DNAPTTH   (SEQ ID NO:3)  ................CT................................C...T....C  1820

MAJORITY                 GTTGGTGGCCTGGACTATAGCCAGATAGAGCTCCGGGTCCTGGCCCACCTCTCCGGGGACCGAGAACCTG

DNAPTAQ                  A..............................A....G.........C.............  1884
DNAPTFL                  .C.....T.T......C......T.........T.................A........  1881
DNAPTTH                  ......................C.........C.....C....................  1890

MAJORITY                 ATCCGGGTCTTCCAGGAGGGGAGGGACATCCACACCCCAGACGGGCCAGCTGGATGTTCGGCGTCCCCGCGG

DNAPTAQ                  ...........C.............GG.............G...............TT....C..  1954
DNAPTFL                  ...........T..................................  1951
DNAPTTH                  ....A..................A.....................................  1960

MAJORITY                 AGGCCGTGGACCCCCTGATGCCCGGCGGGGGGGGGCCAAGACCATCAACTTCGGGGTCCTCTACGGGCATGTCCCC

DNAPTAQ                  .A.GG..A....T.....................G...................  2024
DNAPTFL                  ............................GG.G..........C...........  2021
DNAPTTH                  ..........................................  2030

MAJORITY                 CCACCGCCTCTCCCAGGAGCTTGCCATCCCCTACGGAGGAGGGCGGTGGCCTTCATTGAGCGGCTACTTCCAG

DNAPTAQ                  ..........A.......T.........CCA.............T...  2094
DNAPTFL                  .......GG........T.......................  2091
DNAPTTH                  ...TA.G................................T.A.............A  2100
```

FIGURE 2 (cont'd)

```
MAJORITY (SEQ ID NO:7)  AGCTTCCCCAAGGTGCGGGGCCTCGATTGAGAAGACCCTGGAGGAGGGCAGGAGGCGGGGGGTACGTGGGAGA

DNAPTAQ (SEQ ID NO:1)   .........................GG.......C...........C.CC..........T..........   2164
DNAPTFL ( SEQ ID NO:2)  ........A...........A.A...........G...A.....C................A.          2161
DNAPTTH (SEQ ID NO:3)   ......................................................................   2170


MAJORITY                CCCTCTTCGGCGGGCGGGGGGCCTACGTGCCCGACCTCAACGCCCGGGTGAAGAGCGTGCCGGGAGGCGGCGGA

DNAPTAQ                 .................C..............A.....AG.G.........................C...    2234
DNAPTFL                ..........................T......................C..........             2231
DNAPTTH                ........AA.AA....................................CA...........C....        2240


MAJORITY                GCGGCATGGCCTTCAACATGCCCGTCCAGGGCACCGCGGCCCGCCGACCTCATGAAGCTGGCCATGGTGAAGCTC

DNAPTAQ                ..............................G......................T...............     2304
DNAPTFL                .......................................................CG...T             2301
DNAPTTH                ..........................................................C...             2310


MAJORITY                TTCCCCCGGCTXCAGGAAATGGGGGCCAGGATGCTCCTXCAGGTCCACGACGAGCTGGTCCTCGAGCCCC

DNAPTAQ                ......A....GG......................T......................            2374
DNAPTFL                ......T.....C........TT.G............G...........                      2371
DNAPTTH                ...C.C.G..G.......C.C......CC...G...........C..........               2380


MAJORITY                CCAAAGAGCGGGGGGGGGGAGGCXGGTGGCCCGCCTTTGGGCCAAGGAGGTCATGGAGGGGGTCTATCCCCTGGCCCGT

DNAPTAQ                .A....A..........CC.....G..........G...................            2444
DNAPTFL                ....G.C......AG...AG.....GG.....CAG.....                           2441
DNAPTTH                ..C..C.......A...G.........C.....AA.C....                          2450
```

FIGURE 2 (cont'd)

```
MAJORITY (SEQ ID NO:7)  GCCCCTGGAGGTGGAGGTGGGGATGGGGGAGGACTGGCTCTCCGCCAAGGAGTAG

DNAPTAQ (SEQ ID NO:1)   .............A...........................GA    2499
DNAPTFL (SEQ ID NO:2)   .............CC..........................       2496
DNAPTTH (SEQ ID NO:3)   ....................T...........GT...          2505
```

FIGURE 3

```
MAJORITY (SEQ ID NO:8)   MX A ML PLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEPVQAVYGFAKSLLKALKEDG·DAVXVVFDAK

TAQ PRO (SEQ ID NO:4)    .......RG....................H.............................I........   69
TFL PRO (SEQ ID NO:5)    ....................................................V.V........       68
TTH PRO (SEQ ID NO:6)    .....E..............................YK..F.........                    70


MAJORITY                 APSFRHEAYEAYKAGRAPTPEDFPRQLALIKELVDLLGLXRLEVPGYEADDVLATLAKKAEKEGYEVRIL

TAQ PRO                  .......GG.......................A.............S...................     139
TFL PRO                  ...................V.............F.......R.......                      138
TTH PRO                  .......................FT.......                                      140


MAJORITY                 TADRDLYQLLSDRIAVLHPEGYLITPAWLWEKYGLRPEQWVDYRALXGDPSDNLPGVKGIGEKTAXKLLX

TAQ PRO                  .....K..............D.A...T.E........R.E                               209
TFL PRO                  ........H........Y........A...I.....QR.IR                             208
TTH PRO                  ...V.V.....H..E........F.V.......L...K                                210


MAJORITY                 EWGSLENLLKNLDRVKP·XXREKIXAHMEDLXLSXXLSXVRTDLPLEVDFAXRREPDREGLRAFLERLEF

TAQ PRO                  .....A.....L...AI....L...D.K..WD.AK.........K...R.                     278
TFL PRO                  .....FQH.Q...SL..LQ.G..A.A..RK..Q.H.....GR.T.NL...                    277
TTH PRO                  .....ENV...K.L...R.LE..R........L.QG.                                 280


MAJORITY                 GSLLHEFGLLEXPKALEEAPYPPPEGAFVGFVLSRPEPMYAELLALAAARXGRVHRAXDPLXGLRDLKEV

TAQ PRO                  .....S......D......G..........PE.YKA.....A                            348
TFL PRO                  .....G.A.....WE.L...Q..R...G.                                         347
TTH PRO                  .....A.AP.......K..C.D....A..A..K.....                                350
```

FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)  RGLLAKDLAVLALREGLDLXPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEDAGERALLSERLFXNLXX

TAQ PRO  (SEQ ID NO:4)  ........S.....G.P...................E....A.........A....A..WG  418
TFL PRO  (SEQ ID NO:5)  ....I.........F.E...................E....A.........QT.KE       417
TTH PRO  (SEQ ID NO:6)  ........S......V..........AH........................HR..LK     420

MAJORITY                RLEGEERLLWLYXEVEKPLSRVLAHMEATGVRLDVAYLQALSLEVAEEIRRLEEEVFRLAGHPFNLNSRD

TAQ PRO                 ...........R...R...A.........R.............A....A.            488
TFL PRO                 ...K...E.......R.........EA.V.Q.                             487
TTH PRO                 ...K...H...............L.                                    490

MAJORITY                QLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKNTYIDPLPXLVHPRTG

TAQ PRO                 ...................S......D.I.                               558
TFL PRO                 ...DR..............A....K.                                   557
TTH PRO                 ...R..L..Q.............H.......V.....S.                      560

MAJORITY                RLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFVAEEGWXLVALDYSQIELRVLAHLSGDENL

TAQ PRO                 ...................I......L.                                 628
TFL PRO                 ..................V...V.                                     627
TTH PRO                 .........A.A...........A...                                  630

MAJORITY                IRVFQEGRDIHTQTASWMFGVPPEAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIPYEEAVAFIERYFQ

TAQ PRO                 ......E.......R...............Q.                             698
TFL PRO                 ...........S..G......G..S.                                   697
TTH PRO                 .........K..................V.                               700
```

FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)   SFPKVRAWIEKTLEEGRRRGYVETLFGRRRYVPDLNARVKSVREAAERMAFNMPVQGTAADLMKLAMVKL

TAQ PRO  (SEQ ID NO:4)   ..................................E...................... 768
TRL PRO  (SEQ ID NO:5)   ..Y......G.............................................R. 767
TTH PRO  (SEQ ID NO:6)   ...............K......................................... 770


MAJORITY                 FPRLXEMGARMLLQVHDELVLEAPKXRAEXVAALAKEVMEGVYPLAVPLEVEVGXGEDWLSAKEX

TAQ PRO                  ....E.............E..A..R.........I................. 833
TRL PRO                  ....Q.L..........D...R......W..Q.....L............. 831
TTH PRO                  ....R............QA...E........A..KA.......M..........G 835
```

FIGURE 4

FIGURE 5

Genes for Wild-Type and Pol(-) DNAPTฦ
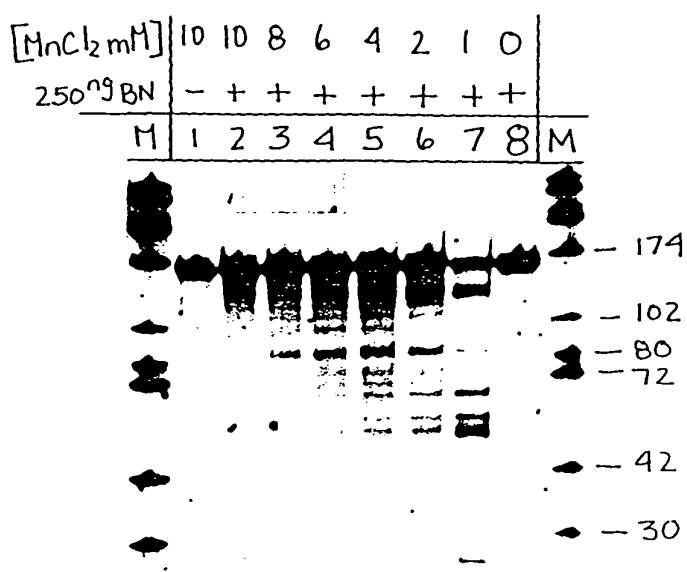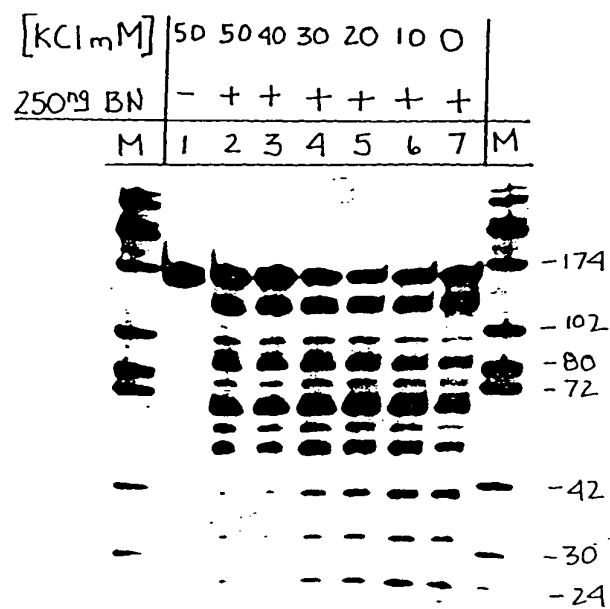
FIGURE 6

Figure 6

FIGURE 7

FIGURE 8

FIGURE 9

A

	1	2	3	4	5	6
DNAP-T:	−	+	+	+	+	+
MgCl$_2$:	+	−	+	+	+	+
dNTPs:	+	−	+	−	+	−
Primers:	+	−	+	+	−	−

◂25
◂21
◂11

B

°C: 0 37 55 72

◂25
◂11

25

19

FIGURE 10

A   B

FIGURE 11

FIGURE 12

FIGURE 13

A

Substrate RNA (46 nt)

```
    AAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCC 3'
    3'CGTACGGACGTCCAGCTGAGATCTCCTAGG 5'
                    30-0
```
15 nt

5'

B

DNAP: − + +
30-0: − − +

▸46

▸31

▸15

FIGURE 14

-35
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG
                    -10   RBS

MetAsnSer...
ATGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCC
    EcoRI   SstI   KpnI   SmaI   BamHI   XbaI   SalI   PstI   SphI   HindIII

ptac   lacZ

pTTQ118
4563 b.p.

lac IQ

Ampr

rrnBt

RBS: Ribosome binding site

ptac: Synthetic tac promoter

lac IQ: Lac repressor gene

lacZ: Beta-galactosidase alpha fragment

rrnBt: E. coli rrnB transcription terminator

08/520946

FIGURE 15

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTT
Bgl II   T7 Promoter   XbaI

MetAlaSer...
AACTTTAAGAAGGAGATATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGATCCGGCT
RBS   NdeI   BamHI

BamHI
NdeI
Bgl II
Tφ
Pφ10
pET-3c
4.6 kb
Amp^r
ori

Pφ10: Bacteriophage T7 φ10 promoter
Tφ: T7 φ Terminator

RBS: Ribosome binding site

FIGURE 16

A

Cleavage site → Substrate Strand 3'
5' arm
Pilot Oligonucleotide 5'
3' arm

B

Cleavage site → Substrate Strand 3'
5' arm
Pilot Oligonucleotide 5'
primer
3' arm

C

Cleavage site → Substrate Strand 3'
5' arm
3'
Pilot Oligonucleotide 5'

D

Cleavage site → substrate strand 3'
5' arm
3' Pilot Oligonucleotide 5'

E

Cleavage Sites
a b
5' arm
TAATACGACTCACTATAGGGAGACCGGAATTCGAGCTCGC C C
G
ATTATGCTGAGTGATATCCCTCTGGCCTTAAGCTCGAGCG G
primer
3' arm
5' GATTCGATTAGCGAC...
CTAAGCTAATCGCTG...

FIGURE 17

FIGURE 18

				4b	4c	4d 4e	4f
Enzyme	Taq	—	Taq	+	+	+	+
Primed M13	—	+	+	+	+	+	+

Unincorporated ^{32}P dCTP →

Incorporated ^{32}P dCMP →

Enzyme

Primed M13

FIGURE 19

A

(32P) ☆ TAATACGACTCACTATAGGGAGAC CGGAAT T C
5'

Sites of Cleavage
with a
gap of 6 nL
60% ▼ ▼ 40%

5'
GATTTAGGTGACACTATAG   3'
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA A G
3'

B

"4d"   "Ab"
No   (2 pr.   Unmodified
Pol.   mutation
Activity small   DNAn Taq
        activity

1 2 3 4 5 6 7 8

Cᴹᵉ   , Tᵃᵍ/   Tᵃᵍ /c.   d N T P
  +   +   - +

84 nt —— ← hairpin test molecule

← conversion to double stranded.
(complete extension of
primer)

} Multiple bands
} caused by polymerization

desired →
product
21 nuc.

└ some aberrant cleavage with
"4b" because of residual
polymerase activity.

FIGURE 20

A

```
5'
--CGGACGAACAAGCGAGACAGCGACACAG|        A
                            GTACC C        A-Hairpin
"Tau"                       CATgG A
CAAAGACGacACAgCAgAgAgAACGGAGAA^        T
3'
```

Predicted cleavage sites

```
5' GTTTCTCctgTGTcGTcTcTcTTGCCTCTT|     A
                            GTAcc T        T-Hairpin
"Alpha"                     CATGG G
----CTGCTTGTTCGCTCTGTCGCTGTGTC^       T
3'
```

B   Sequence of alpha primer :

5' GAC GAA.CAA.CCG AGA CAG CG 3'

C

```
          5' ACACAG|       A
                  GTACC C
"Tau"             CATgG A
3' CAAAGACGacACAgCAgAgAgAACGGACAA^   T
   Cleaved A-Hairpin
```

```
                    5' CCTCTT|       A
                             GTACC T
"Alpha"                      CATGG G
3'--CTGCTTGTTCGCTCTGTCGCTGTGTC^    T
   Cleaved T-Hairpin
```

D

Top = T-Hairpin
Bottom = A-Hairpin

```
                              Rsa I
                               Mnl I
                                Nla III
                                 HgiC I
                                  Nla IV
                                   Rsa I
         BsmAI                      Kpn I              BsmAI
GTTTCTGCTGTGTCGTCTCTCTTGCCTCTTGTACCATGTGGTACCTGTGTCGCTGTCTCGCTTGTTCGTC  72
CAAAGACGACACAGCAGAGAGAACGGAGAACATGGTACACCATGGACACAGCGACAGAGCGAACAAGCAGGC
```

FIGURE 21

Ban II
Sst I
Asp 718
Ava I
Kpn I
Xma I
Sma I
Bam HI XI

EcoR I

CGCCAGGGTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTC

GCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCACTTAACATTATGCTGAGTGATATCCCGCTTAAGCTCGAGCCATGGGCCCCTAGGAG

—— 47 Forward

T7

Pilot 30-0

Sal I
Acc I
Hinc II

Pst I
BspM I
Sph I
Hind III

TAGAGTCCGACCTGCAGGCAGCTGCAAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTA

ATCTCAGGCTGGACGTCCGTACGTTCGAACTCATAAGATATCACAGTGGATTTATCGAACCGCATTAGTACCAGTAGTATCGACAAAGGACACACTTTAACAAT

—— Pilot 30-0

—— SP6

Z

-48 Reverse

TCCGCTCACAATTCCACACAACATACGA
                            228

AGGCGAGTGTTAAGGTGTGTTGTATGCT

-48 Rev

206

FIGURE 22A

1   5'
         Target Nucleic Acid (206 nt)   3'

85 nt

2
     5'
cleavage site
Target Nucleic Acid (206 nt)   3'
3' |||||||||||||||||||| 5'
Pilot Oligonucleotide

85 nt

3
     5'
cleavage site
Target Nucleic Acid (206 nt)   3'
|||||||||||||||||||||
3'   Pilot Oligonucleotide   5'

FIGURE 22B

FIGURE 23

FIGURE 24

CDR Bead
Pilot
Cleavase

FIGURE 25

FIGURE 26

A →

B ⇄

A   B

-206

• = ^{32}P

FIGURE 27

FIGURE 28A

$*$ = ^{32}P 5' terminal phosphate

FIGURE 28B

M 1 2 3 4 5 6

—21

—1

FIGURE 29

Wild-type Substrate   Mutant Substrate

1

Denature

5' _____ A ___ 3'   5' _____ G ___ 3'

2

Renature

5' _____ A __ A __ A __ 3'   5' _____ A __ A __ G __ 3'

3

Add cleavage agent

5' ►___ ►__ ►__ A __ 3'   5' ►___ ►__ ►__ ►__ G __ 3'

► = cleavage site

4

Resolve reaction products

5

Detect unique cleavage "fingerprint"
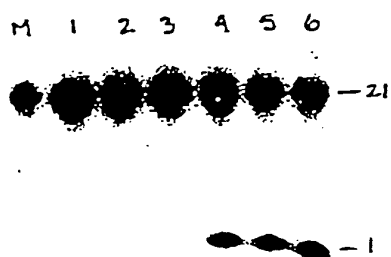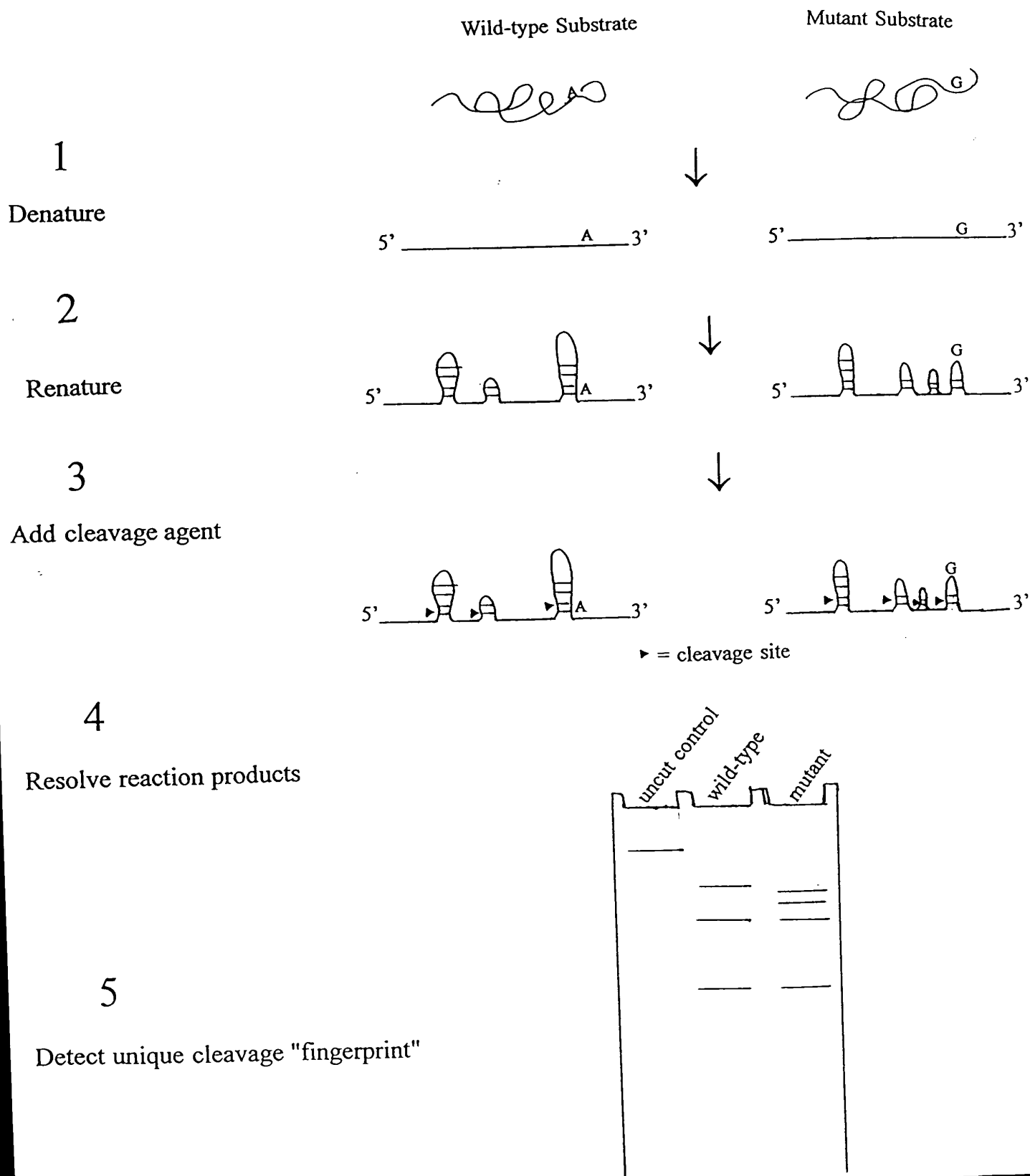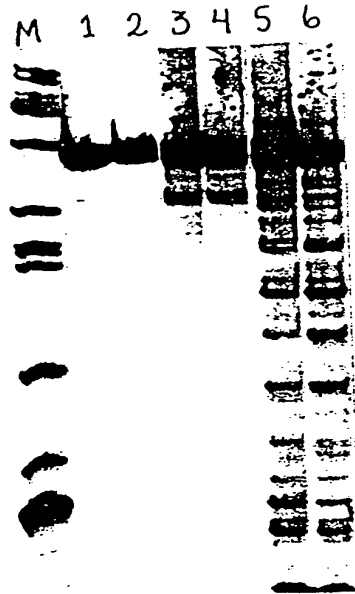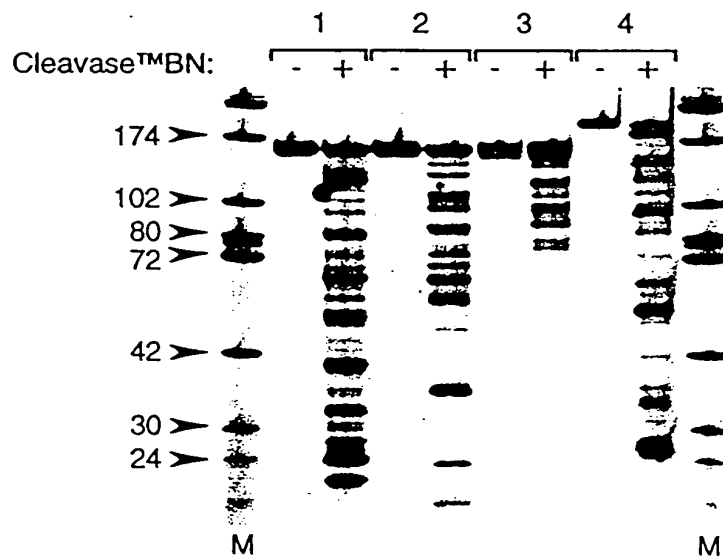
FIGURE 30

FIGURE 31

FIGURE 32

FIGURE 33

FIGURE 34

| [MnCl₂ mM] | 10 | 10 | 8 | 6 | 4 | 2 | 1 | 0 | | |

$[MnCl_2 \, mM]$: 10 10 8 6 4 2 1 0

$250^{ng} BN$: − + + + + + + +

M | 1 2 3 4 5 6 7 8 | M

— 174
— 102
— 80
— 72
— 42
— 30

FIGURE 35

FIGURE 36

Time (min.):   10 <.1 1 5 10
Cleavase™BN: - + + + +

uncut (157)

102
80

42

30
24

M   M

FIGURE 37

Temperature (°C): 55 80 55 60 65 70 75 80
Cleavase™BN: - - + + + + + +

uncut (157) >

102 >

80 >

M M

FIGURE 38

Cleavase™BN (ng):   - 10 50 100 250

uncut (157)

102
80

42

30
24

M   M
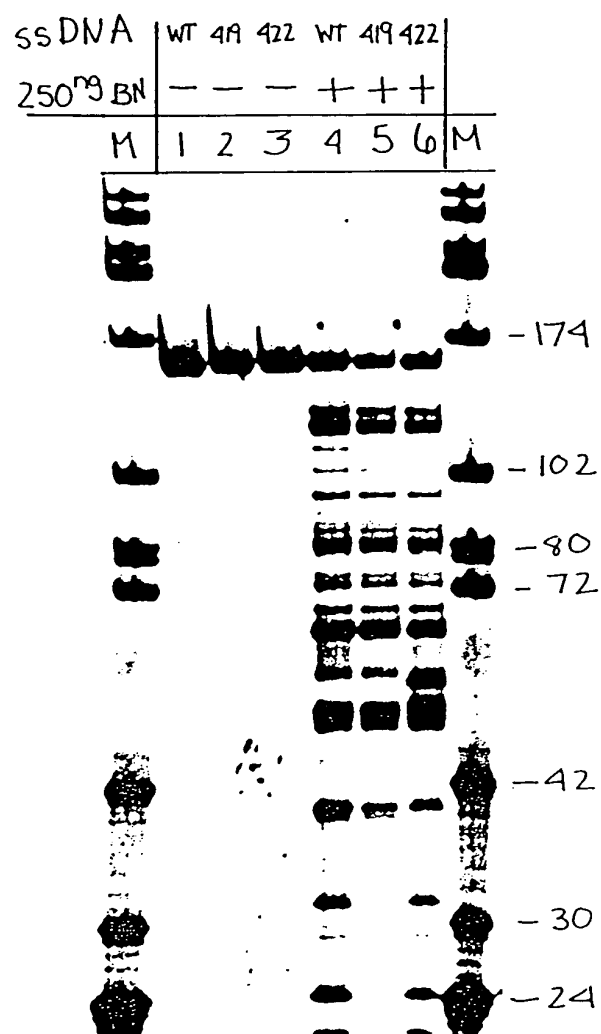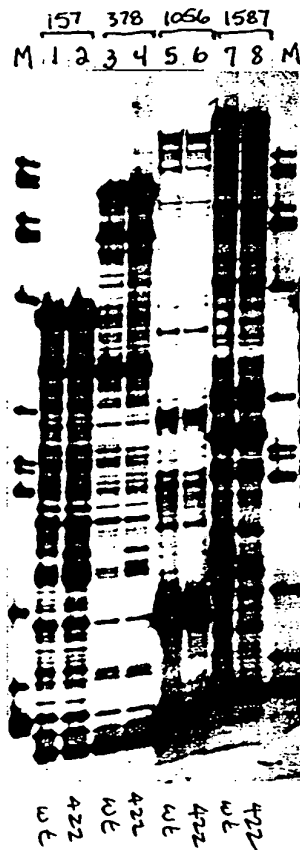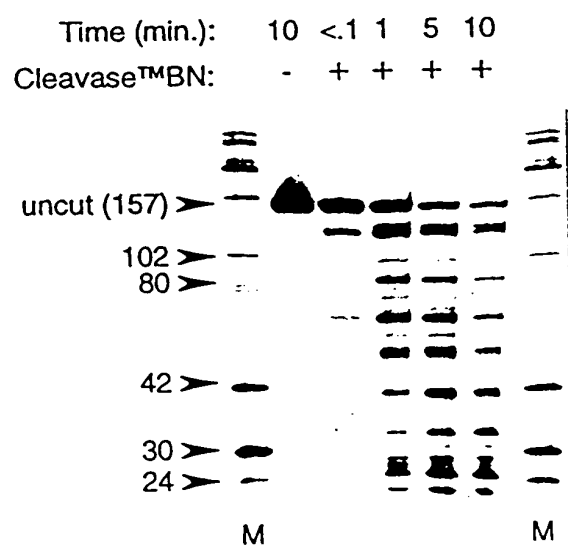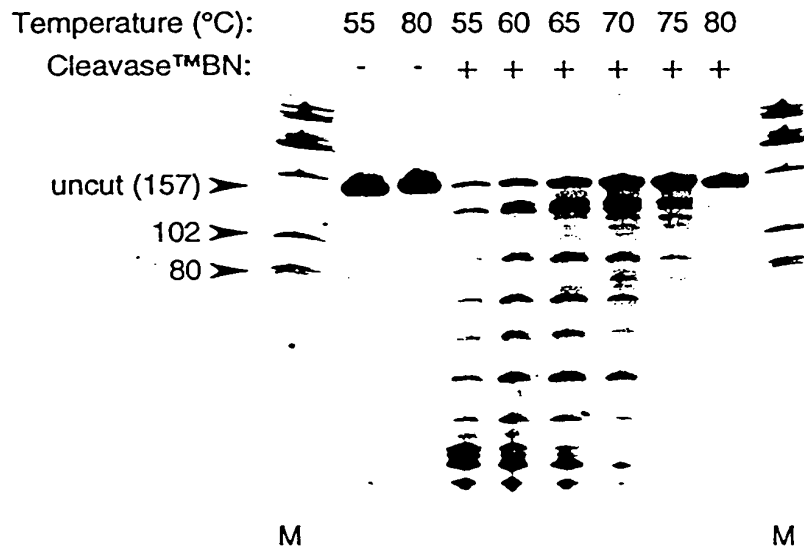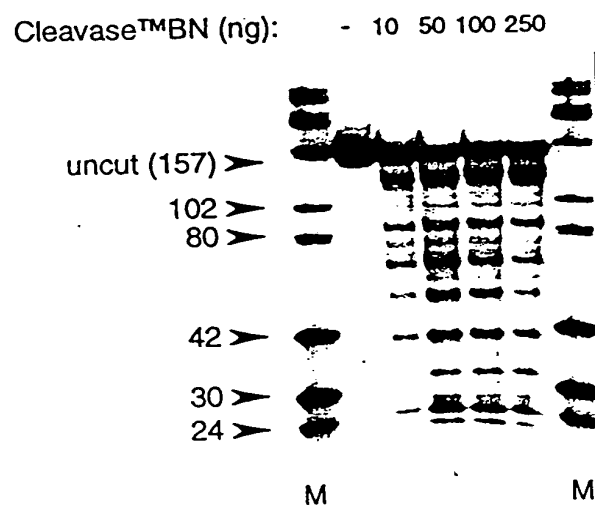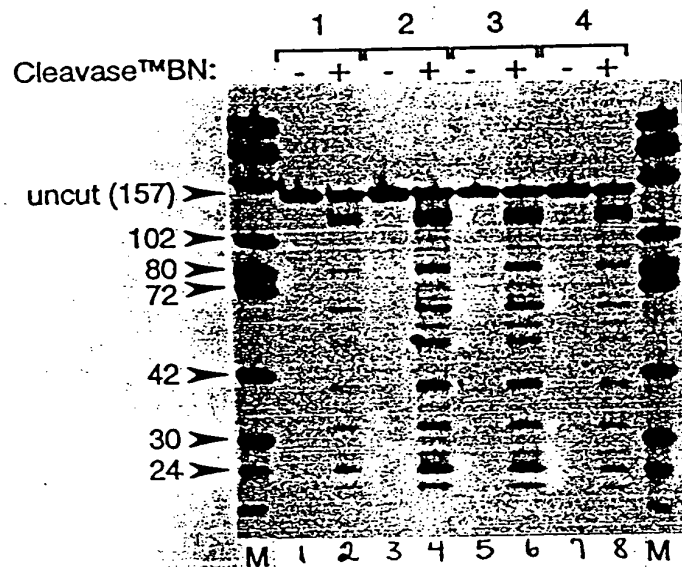
FIGURE 39

FIGURE 40

strand	5' BIOTIN SENSE STRAND						5' FLUORESCEIN ANTI-SENSE strand					
ss DNA	Wt	419	422	WT	419	422	WT	419	422	WT	419	422
250 ng BN	−	−	−	−	+	+	+	+	+	+	−	−	−	−
M	1	2	3	4	5	6	7	8	9	10	11	12
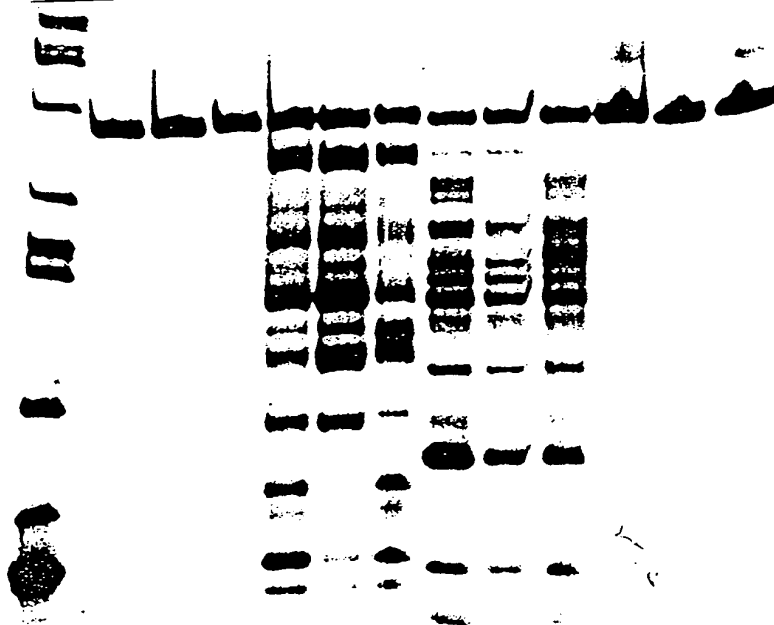
FIGURE 41
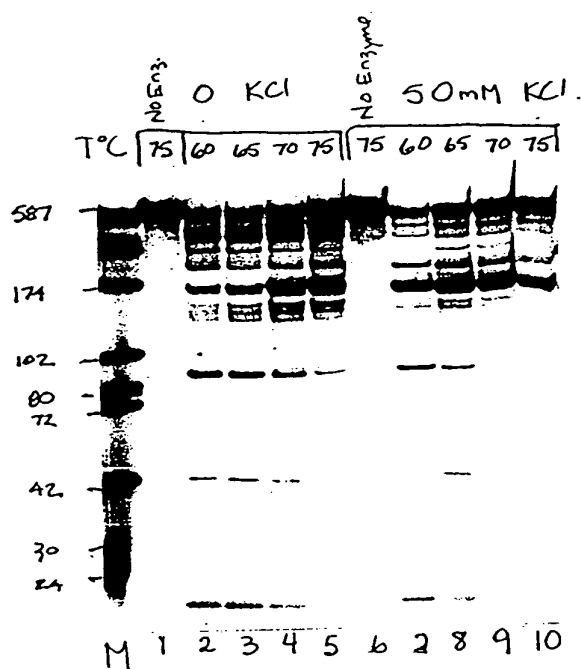
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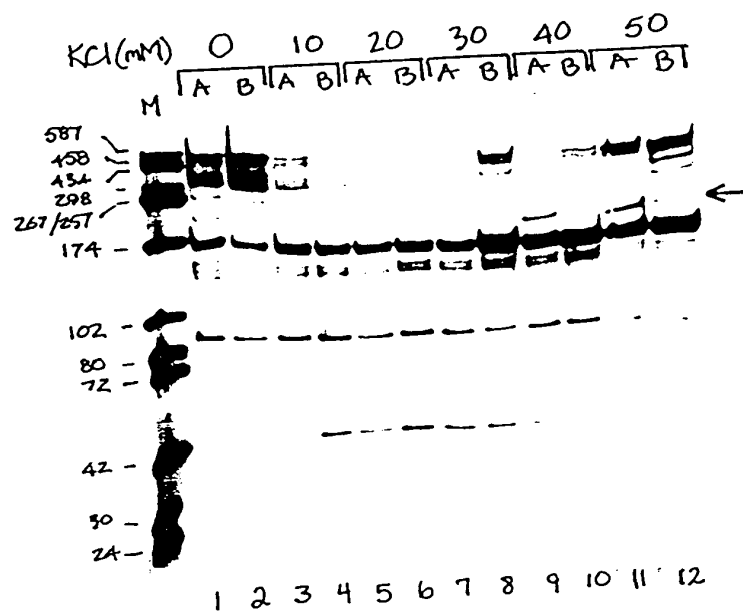
08/520946

FIGURE 43

FIGURE 44
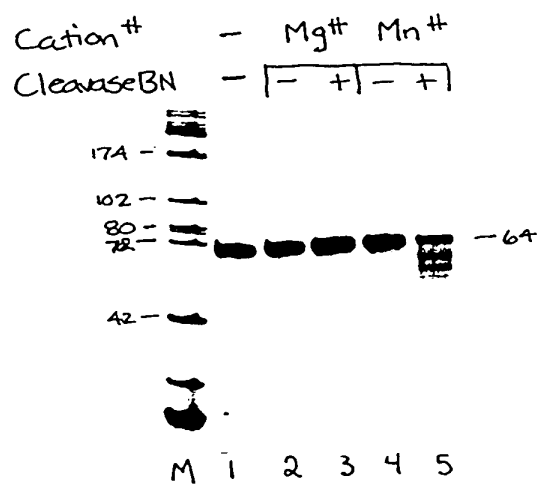
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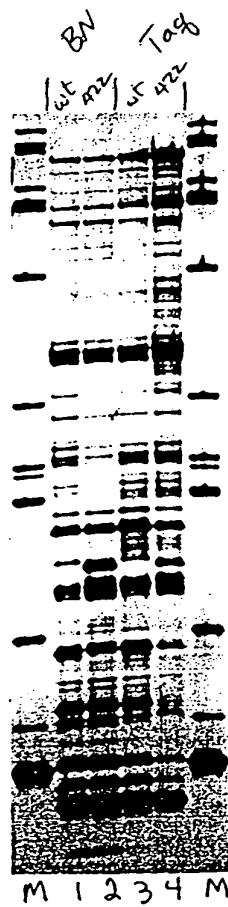
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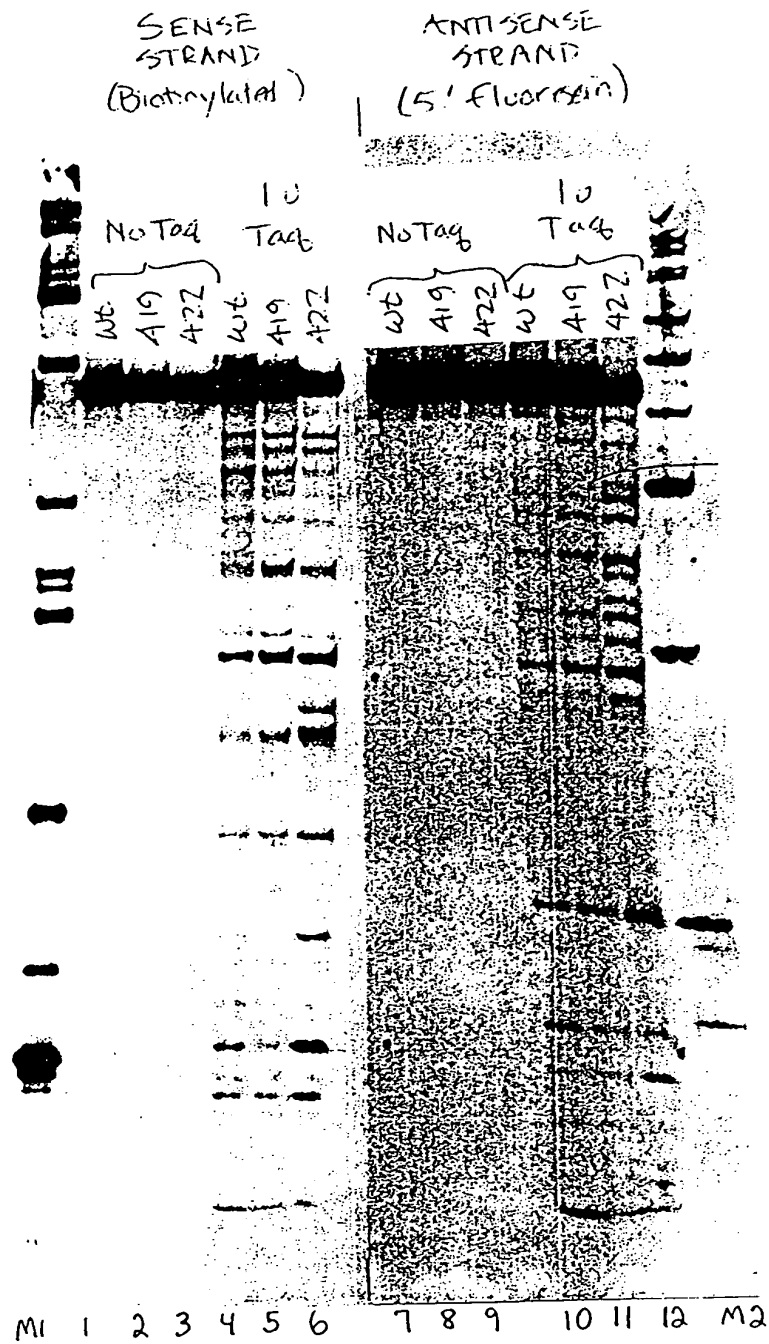
FIGURE 47

FIGURE 48

wild type
R422Q
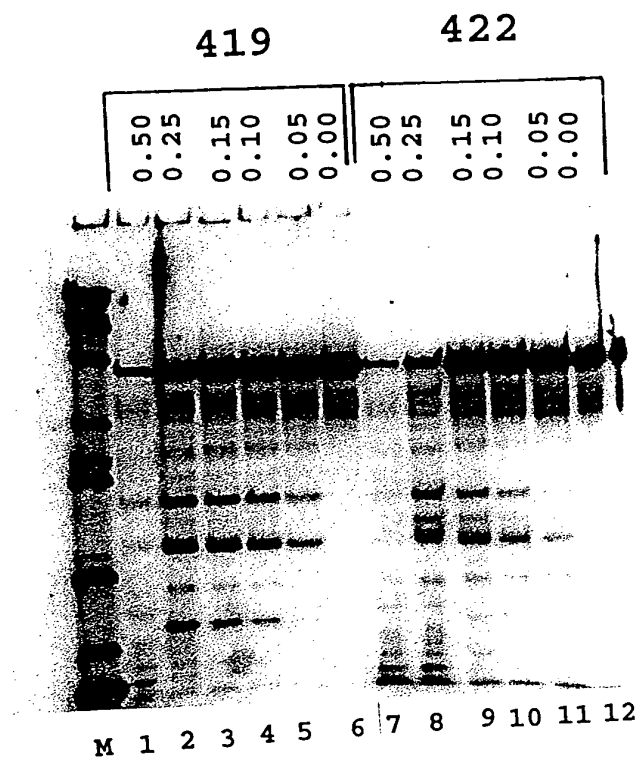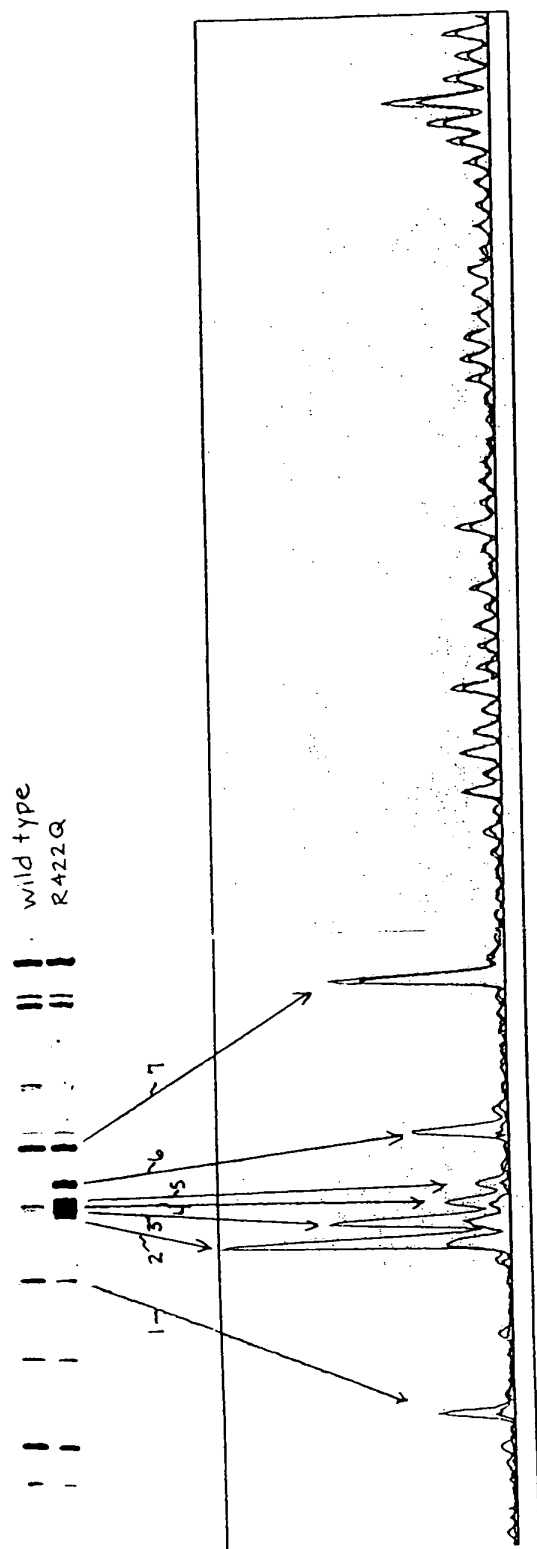
FIGURE 49

```
                            50                                                100

L.100.8-1      5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGGACTTTCCACAAGGGG ATGTTACGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO:76) 3'CCGACTGTTCTTCCTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC  TACAATGCCCTCGGCCAGCCCTTGCGGGTGAGAGA

L.46.16-10     5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGGACTTTCCACAAGGGG ATGTTATGGGGAGG-----------AGCCGGTCGGGAACACCCACTTCT
(SEQ ID NO:77) 3'CCGACTGTTCTTCCTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC  TACAATACCCCTCC-------TCGGCCAGCCCTTGTGGGTGAAAGA

L.46.16-12     5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGGACTTTCCACAAGGGG ATGTTATGGGGAGG-----------AGCCGGTCGGGAACACCCACTTCT
(SEQ ID NO:78) 3'CCGACTGTTCTTCCTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC  TACAATACCCCTCC------TCGGCCAGCCCTTGTGGGTGAAAGA

L.19.16-3      5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGGACTTTCCACAAGGGG ATGTTACGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCCCTCTCT
(SEQ ID NO:79) 3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC  TACAATGCCCTCGTCCCTGAAAGGTGTTCCCC

L.CBM/251      5'GGCTGACAAGAAGGAAACTCGCTGAAACAGGAGGGTACTTTCCACAAGGGG ATGTTACGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTTTCT
(SEQ ID NO:80) 3'CCGACTGTTCTTCCTTTGAGCGACTTTGTCGTCCCTGAAAGGTGTTCCCC  TACAATGCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAAAGA

L.36.8-3       5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGGACTTTCCACAAGGGG ATGTTACGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO:81) 3'CCGACTGTTCTTCCTTTGAGCGACTCGTCGTCCCTGAAAGGTGTTCCCC  TACAATGCCCTCGTCCCCAGCCCCTTGCGGGTGAGAGA


                           150                                               200

L.100.8-1      5'TGATGTATAAATATCACTGCATTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
               3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.46.16-10     5'TGATGTATAAATATCACTGCATTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
               3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.46.16-12     5'TGGTGTATAAATATCACTGCATTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
               3'ACCACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.19.16-3      5'TGATGTATAAATATCACTGCATTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
               3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.CBM/251      5'TGATGTATAAATATCACTGCATTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
               3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.36.8-3       5'TGATGTATAAATATCACTGCATTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
               3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGATCCTCCAAGAGAGGTCGTGATCGTCCATC
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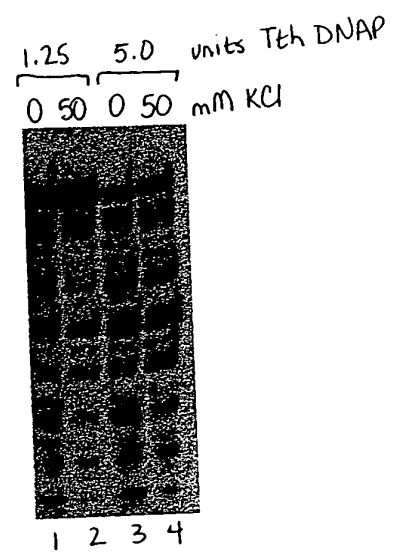
FIGURE 49

250   300

L.100.8-1
(SEQ ID NO:76)
5' AGCCTGGGTGTTCCCTGCTAGACTCTTCACCAGCACTTGGCCGGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
3' TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCACCGAGGTGCGAACGAACGAATTCTGGAGAAGTTATTTCGACGG

L.46.16-10
(SEQ ID NO:77)
5' AGCCTGGGTGTTCCCTGCTAGACTCTTCACCAGCACTTAGCCAGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
3' TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAATCGGTCACGACCC GTCTCACCGAGGTGCGAACGAATTCTGGAGAAGTTATTTCGACGG

L.46.16-12
(SEQ ID NO:78)
5' AGCCTGGGTGTTCCCTGCTAGACTCTTCACCAGCACTTGGCCAGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACGAACGAACGAATTCTGGAGAAGTTATTTCGACGG
3' TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGTCACGACCC GTCTCACCGAGGTGCGAACGAATTCTGGAGAAGTTATTTCGACGG

L.19.16-3
(SEQ ID NO:79)
5' AGCCTGGGTGTTCCCTGCTAGACTCTTCACCAGCACTTGGCCGGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGCCCTCTTCAATAAAGCTGCC
3' TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCACCGAGGTGCGAACGAATTCTGGAGAAGTTATTTCGACGG

L.CEM/251
(SEQ ID NO:80)
5' AGCCTGGGTGTTCCCTGCTAGACTCTTCACCAGCACTTGGCCGGTGCTGGG CAGAGTGACTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
3' TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCACTGAGGTGCGAACGAATTCGGGAGAAGTTATTTCGACGG

L.36.8-3
(SEQ ID NO:81)
5' AGCCTGAGTGTTCCCTGCTAAACTCTTCACCAGCACTTGGCCGGTGCTGGG CAGAGGGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
3' TCGGACTCACAAGGGACGATTTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCGCCGAGGTGCGAACGAATTCTGGAGAAGTTATTTCGACGG

|— Hairpin —|

350

L.100.8-1
5' ATTTTAGAAGTAGGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3' TAAAATCTTCATCCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

L.46.16-10
5' ATTTTAGAAGTAAGCCAGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3' TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

L.46.16-12
5' ATTTTAGAAGTAAGCCAGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3' TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

L.19.16-3
5' ATTTTAGAAGTAGGCTAGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3' TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

L.CEM/251
5' ATTTTAGAAGTAAGCTAGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3' TAAAATCTTCATTCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

L.36.8-3
5' ATTTTAGAAGTAGGCTAGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3' TAAAATCTTCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

FIGURE 50

M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 51

M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 52

No enz.

0 10 20 30 40 50 75 100 mM NaCl

M 1 2 3 4 5 6 7 8 9

FIGURE 53

No enz. 0, 10, 20, 30, 40, 50, 75, 100 mM $(NH_4)_2SO_4$

M 1 2 3 4 5 6 7 8 9

FIGURE 54

FIGURE 55

FIGURE 56

1 2 3 4 5 6 7

FIGURE 57

FIGURE 58

FIGURE 59

FIGURE 60
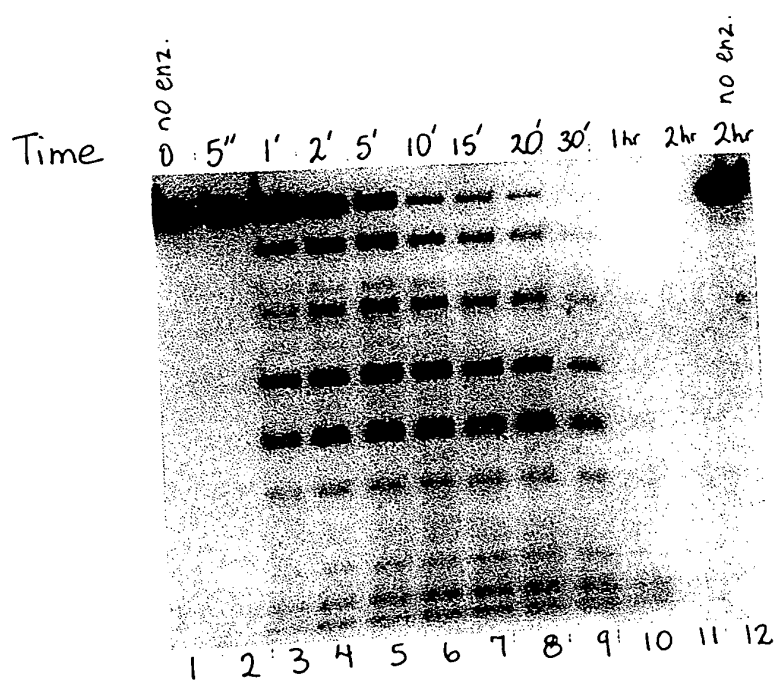
FIGURE 61

FIGURE 62

FIGURE 63

0 , 1 , 5 , 10 , 25 , 50 , 100 , 250 ng Cleavase™ BN

M 1 2 3 4 5 6 7 8

FIGURE 64

FIGURE 65

A

B

8.2   7.2   pH
+ - + -   Cleavase™ BN   - + - +
7.5   7.8

1 2 3 4

1 2 3 4
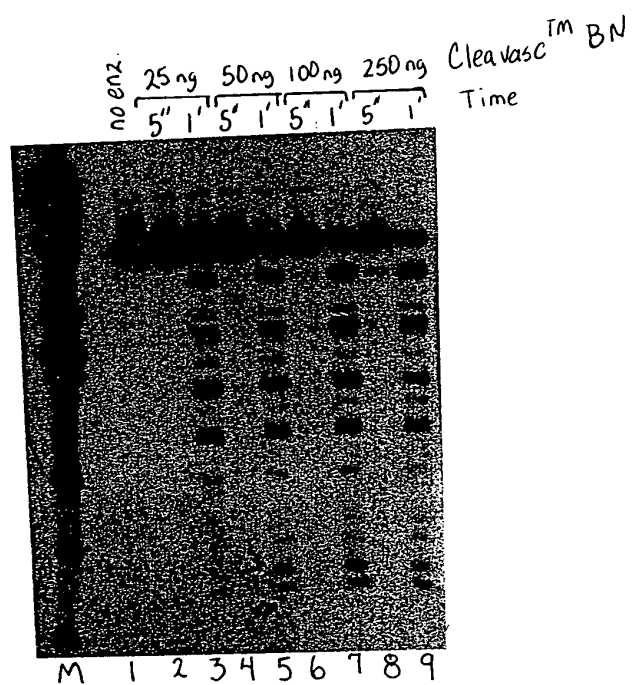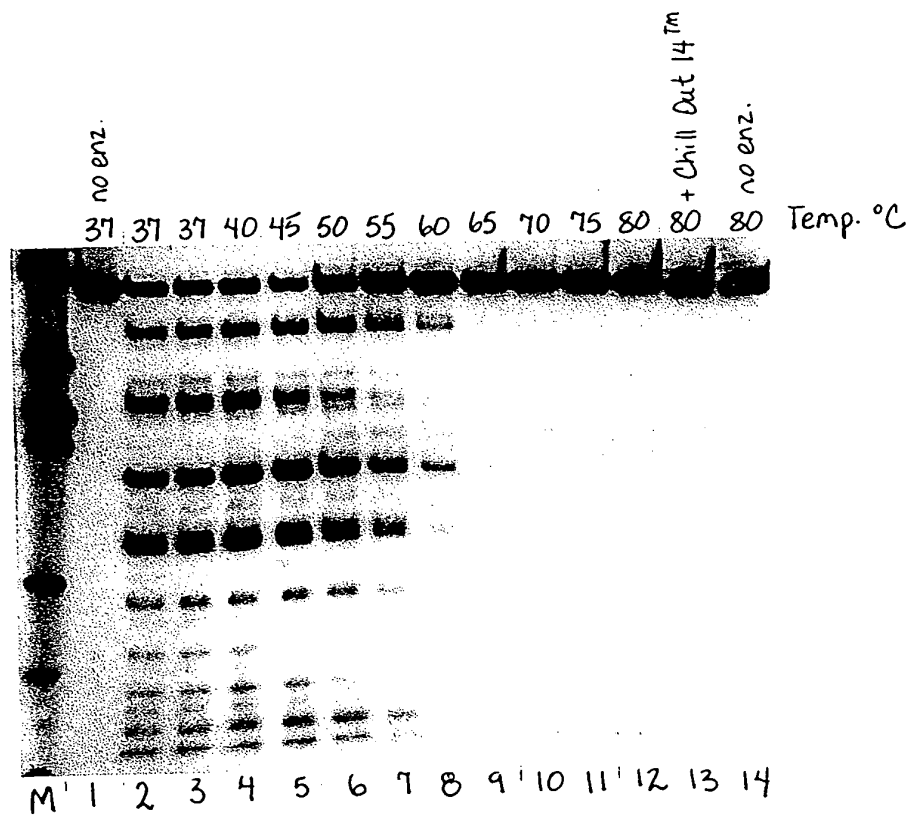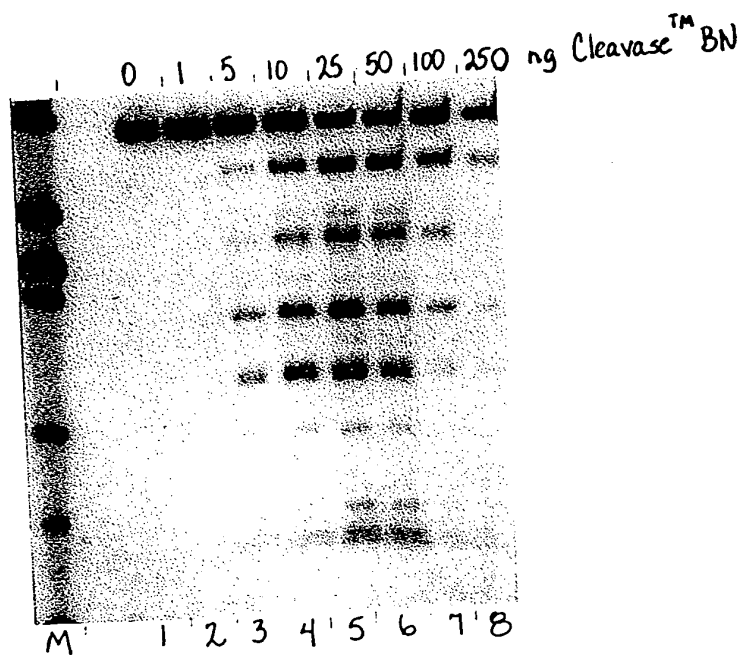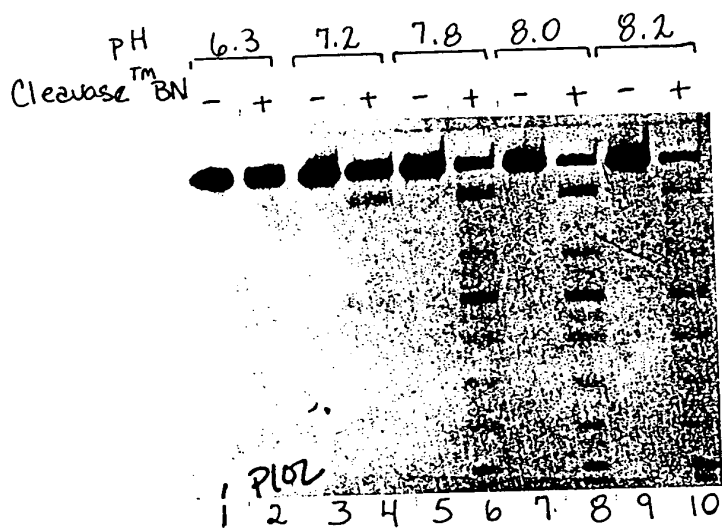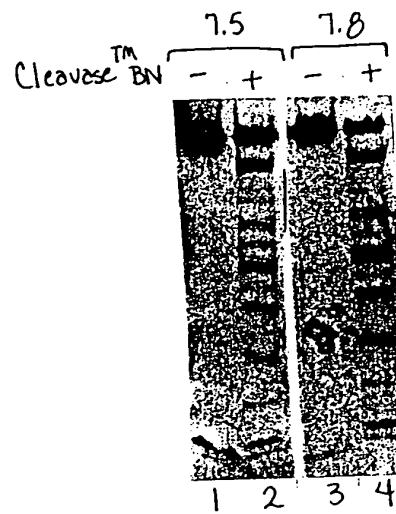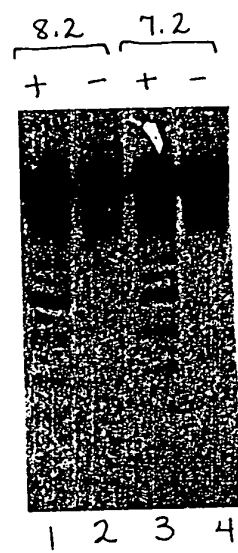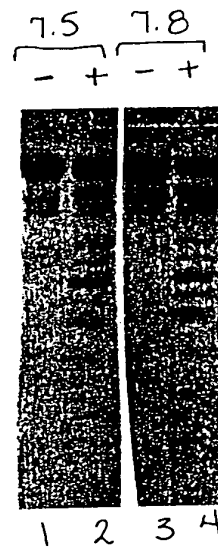
FIGURE 66

FIGURE 67

FIGURE 68

1.25   5.0   units Tth DNAP

0 50 0 50 mM KCl

1 2 3 4

FIGURE 69

FIGURE 70

Tth DNAP: wt, 419, 422 (lanes 1, 2, 3)

E. coli Exo III: 0mM (wt, 419, 422 — lanes 3, 4, 5), 50mM (wt, 419, 422 — lanes 6, 7, 8)

Cleavase™ BN (lane 9)

no enz (lane 10)

KCl

1 2 3 4 5 6 7 8 9 10 11

FIGURE 71

5' cleavage site

3' cleavage site

FIGURE 72

FIGURE 73

174

M 1 2 3 4 5 6

FIGURE 74

A

B

mutant

wt 1 2 3

1 2 3 4 M

Isolate #

4 5 6 7 8

M 1 2 3 4 5 M

FIGURE 75

M 1 2 3 4 5 6 7 8 9

FIGURE 76

FIGURE 77

PCR 1   Generate Fragment Containing Mutation

Add Amplified Fragment to PCR 2

PCR 2

Denature and Amplify

Insert Mutant into Fragment by PCR

Digest and Clone into Vector

Sequence and Archive

PCR Amplify Exons 5-8

CFLP Analysis of Exons 5-8

FIGURE 78

p53 Segment

"upstream PCR"

"downstream PCR"

Combine,
Denature &
Anneal

&

"Recombinant
PCR"

Recombinant p53 segment

FIGURE 79

FIGURE 80

FIGURE 81

FIGURE 82

```
HCV1.1  (SEQ ID NO:121)
HCV2.1  (SEQ ID NO:122)
HCV3.1  (SEQ ID NO:123)
HCV4.2  (SEQ ID NO:124)
HCV6.1  (SEQ ID NO:125)
HCV7.1  (SEQ ID NO:126)

1      CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG  50
HCV1.1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG
HCV2.1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG
HCV3.1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG
HCV4.2 CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV6.1 CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTA  GAGTGTCGTA
HCV7.1 CTGTCTTCAC GCAGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTCGTG

51     CAGCCTCCAG GACCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC  100
HCV1.1 CAGCCTCCAG GACCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV2.1 CAGCCTCCAG GACCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV3.1 CAGCCTCCAG GTCCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV4.2 CAGCCTCCAG GACCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV6.1 CAGCCTCCAG GCCCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV7.1 CAGCCTCCAG GACCCCCCT  CCCGGGAGAG CCATAGTGGT CTGCGGAACC

101    GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-AAA  150
HCV1.1 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-AAA
HCV2.1 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV3.1 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV4.2 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC GTGGATGTAA
HCV6.1 GGTGAGTACA CCGGAATTGC CGGGAAGACT GGGTCCTTTC TTGGAT-AAA
HCV7.1 GGTGAGTACA CCGGAATTGC TGGGTGACC  GGGTCCTTTC TTGGAG-CAA

151    CCCGCTCAAT GCTTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG  200
HCV1.1 CCCGCTCAAT GCTTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV2.1 CCCGCTCAAT GCTTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV3.1 CCCGCTCAAT GCTTGGAGAT TTGGGCGTGC CCCCGCGAGA CTGCTAGCCG
HCV4.2 CCCGCTCAAT GCTTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV6.1 CCCACTCTAT GCCCGGCCAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV7.1 CCCGCTCAAT ACCCAGAAAT TTGGGCGTGC CCCCGCGAGA TCACTAGCCG

201    AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCCT  250
HCV1.1 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCCT
HCV2.1 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV3.1 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV4.2 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV6.1 AGTAGCGTTG GGTTGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV7.1 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT

251    GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC  282
HCV1.1 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV2.1 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV3.1 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV4.2 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV6.1 GCGAGTACCC CGGGAGGTCT CGTAGACCGT GC
HCV7.1 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
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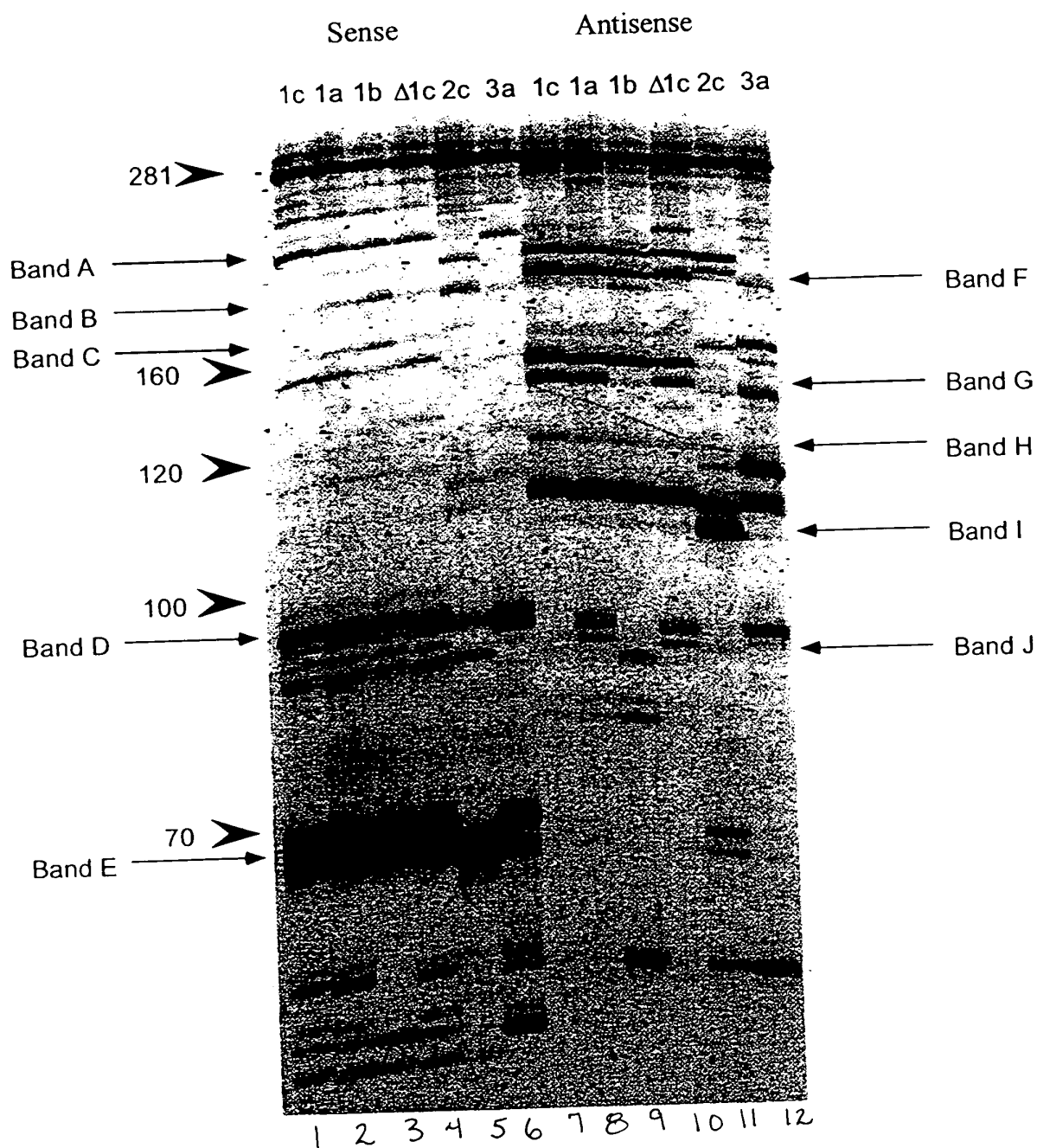
FIGURE 83

Sense   Antisense

1c 1a 1b Δ1c 2c 3a   1c 1a 1b Δ1c 2c 3a

281

Band A →   ← Band F

Band B →

Band C →
160

120   ← Band G

     ← Band H

     ← Band I

100

Band D →   ← Band J

70

Band E →

1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 84

FIGURE 85

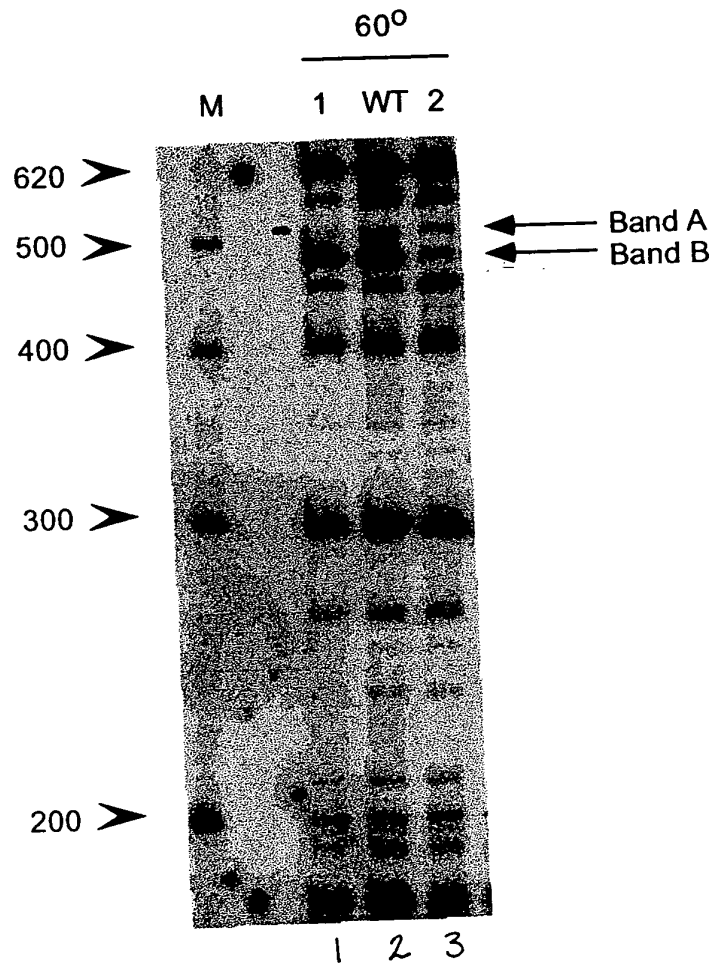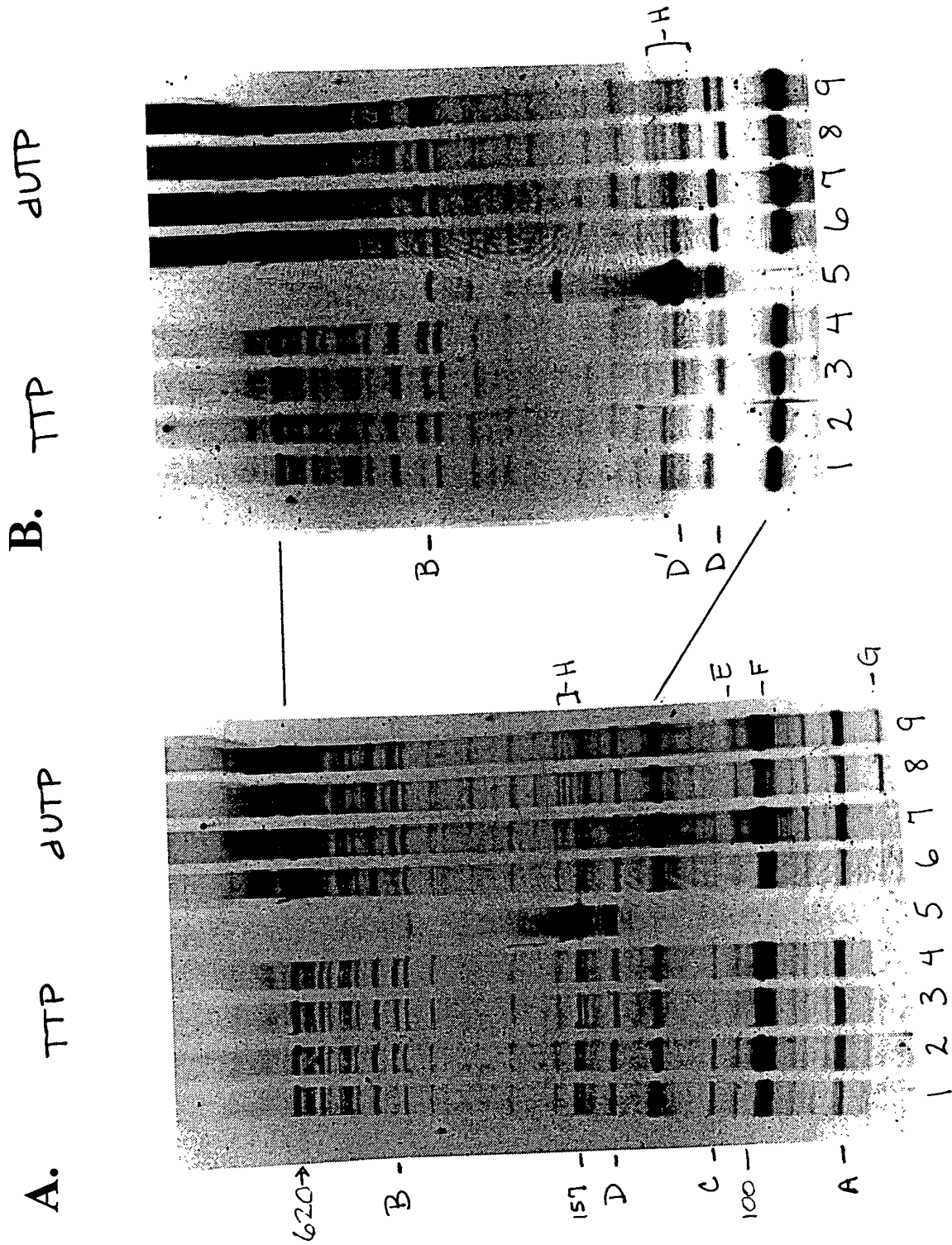**FIGURE 86**
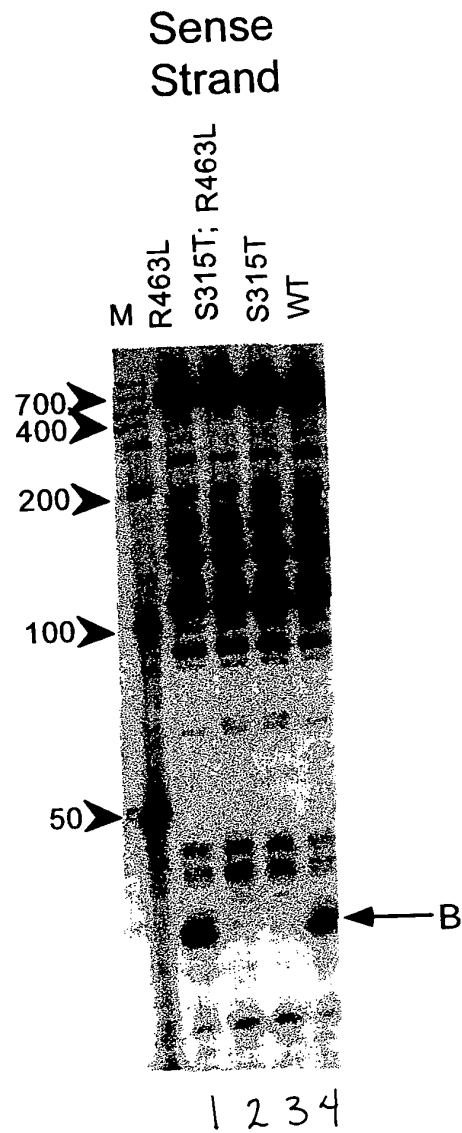
FIGURE 87

Antisense
Strand
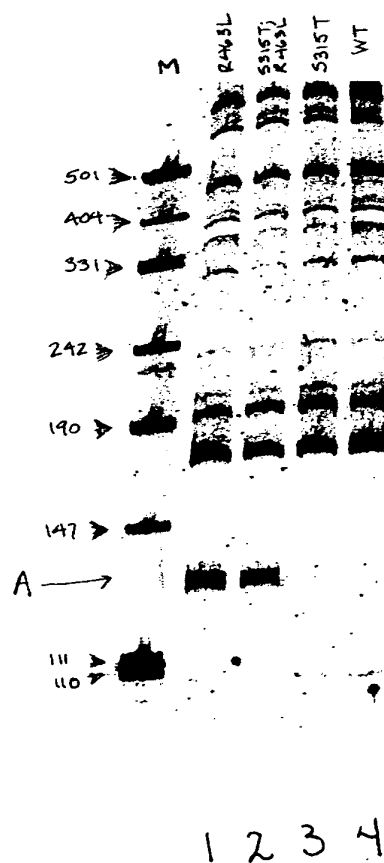
FIGURE 88

Sheet 1/2

```
        10        20        30        40        50        60              1638
        AGA  GTTTGATCCT  GGCTCAG
AAATTGAAGA GTTTGATCAT GGCTCAGATT GAACGCTGGC GGCAGGCCTA ACACATGCAA
TTTAACTTCT CAAACTAGTA CCGAGTCTAA CTTGCGACCG CCGTCCGGAT TGTGTACGTT

        70        80        90       100       110       120             ER10
                                              GGCGGAC  GGGTGAGTAA
GTCGAACGGT AACAGGAAGA AGCTTGCTTC TTTGCTGACG AGTGGCGGAC GGGTGAGTAA
CAGCTTGCCA TTGTCCTTCT TCGAACGAAG AAACGACTGC TCACCGCCTG CCCACTCATT

       130       140       150       160       170       180
TGTCTGGGAA ACTGCCTGAT GGAGGGGGAT AACTACTGGA AACGGTAGCT AATACCGCAT
ACAGACCCTT TGACGGACTA CCTCCCCCTA TTGATGACCT TTGCCATCGA TTATGGCGTA

       190       200       210       220       230       240
AACGTCGCAA GACCAAAGAG GGGGACCTTC GGGCCTCTTG CCATCGGATG TGCCCAGATG
TTGCAGCGTT CTGGTTTCTC CCCCTGGAAG CCCGGAGAAC GGTAGCCTAC ACGGGTCTAC

       250       260       270       280       290       300
GGATTAGCTA GTAGGTGGGG TAACGGCTCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA
CCTAATCGAT CATCCACCCC ATTGCCGAGT GGATCCGCTG CTAGGGATCG ACCAGACTCT

       310       320       330       340       350       360
GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG
CCTACTGGTC GGTGTGACCT TGACTCTGTG CCAGGTCTGA GGATGCCCTC CGTCGTCACC
                                        TGA  GGATGCCCTC  CGTCGTC      1659

       370       380       390       400       410       420
GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG AAGAAGGCCT
CCTTATAACG TGTTACCCGC GTTCGGACTA CGTCGGTACG GCGCACATAC TTCTTCCGGA

       430       440       450       460       470       480
TCGGGTTGTA AAGTACTTTC AGCGGGGAGG AAGGGAGTAA AGTTAATACC TTTGCTCATT
AGCCCAACAT TTCATGAAAG TCGCCCCTCC TTCCCTCATT TCAATTATGG AAACGAGTAA

       490       500       510       520       530       540
GACGTTACCC GCAGAAGAAG CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG
CTGCAATGGG CGTCTTCTTC GTGGCCGATT GAGGCACGGT CGTCGGCGCC ATTATGCCTC

       550       560       570       580       590       600
GGTGCAAGCG TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTT TGTTAAGTCA
CCACGTTCGC AATTAGCCTT AATGACCCGC ATTTCGCGTG CGTCCGCCAA ACAATTCAGT

       610       620       630       640       650       660
GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGCATCTGA TACTGGCAAG CTTGAGTCTC
CTACACTTTA GGGGCCCGAG TTGGACCCTT GACGTAGACT ATGACCGTTC GAACTCAGAG

       670       680       690       700       710       720
GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG TAGAGATCTG GAGGAATACC
CATCTCCCCC CATCTTAAGG TCCACATCGC CACTTTACGC ATCTCTAGAC CTCCTTATGG

       730       740       750       760       770       780
GGTGGCGAAG GCGGCCCCCT GGACGAAGAC TGACGCTCAG GTGCGAAAGC GTGGGGAGCA
CCACCGCTTC CGCCGGGGGA CCTGCTTCTG ACTGCGAGTC CACGCTTTCG CACCCCTCGT
```

FIGURE 88   Sheet 2/2

```
        790         800         810         820         830         840
AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTGCC
TTGTCCTAAT CTATGGGACC ATCAGGTGCG GCATTTGCTA CAGCTGAACC TCCAACACGG


        850         860         870         880         890         900
CTTGAGGCGT GGCTTCCGGA GCTAACGCGT TAAGTCGACC GCCTGGGGAG TACGGCCGCA
GAACTCCGCA CCGAAGGCCT CGATTGCGCA ATTCAGCTGG CGGACCCCTC ATGCCGGCGT


        910         920         930         940         950         960
AGGTTAAAAC TCAAATGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT GTGGTTTAAT
TCCAATTTTG AGTTTACTTA ACTGCCCCCG GGCGTGTTCG CCACCTCGTA CACCAAATTA


        970         980         990        1000        1010        1020
TCGATGCAAC GCGAAGAACC TTACCTGGTC TTGACATCCA CGGAAGTTTT CAGAGATGAG
AGCTACGTTG CGCTTCTTGG AATGGACCAG AACTGTAGGT GCCTTCAAAA GTCTCTACTC


       1030        1040        1050        1060        1070        1080
AATGTGCCTT CGGGAACCGT GAGACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTTGTGA
TTACACGGAA GCCCTTGGCA CTCTGTCCAC GACGTACCGA CAGCAGTCGA GCACAACACT
```

SB-1

```
       1090        1100        1110        1120        1130        1140
                 GC AACGAGCGCA ACCC
AATGTTGGGT TAAGTCCCGC AACGAGCGCA ACCCTTATCC TTTGTTGCCA GCGGTCCGGC
                     ‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
TTACAACCCA ATTCAGGGCG TTGCTCGCGT TGGGAATAGG AAACAACGGT CGCCAGGCCG
```

SB-3
SB-4

```
       1150        1160        1170        1180        1190        1200
                                           ATG ACGTCAAGTC
                                           ATG ACGTCAAGTC
CGGGAACTCA AAGGAGACTG CCAGTGATAA ACTGGAGGAA GGTGGGGATG ACGTCAAGTC
                                               ‾‾‾‾‾‾‾‾‾‾‾‾
GCCCTTGAGT TTCCTCTGAC GGTCACTATT TGACCTCCTT CCACCCCTAC TGCAGTTCAG
```

SB-3
SB-4

```
       1210        1220        1230        1240        1250        1260
ATCATGGCCC TTA
ATCATGGCCC TTACGA
ATCATGGCCC TTACGACCAG GGCTACACAC GTGCTACAAT GGCGCATACA AAGAGAAGCG
‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
TAGTACCGGG AATGCTGGTC CCGATGTGTG CACGATGTTA CCGCGTATGT TTCTCTTCGC


       1270        1280        1290        1300        1310        1320
ACCTCGCGAG AGCAAGCGGA CCTCATAAAG TGCGTCGTAG TCCGGATTGG AGTCTGCAAC
TGGAGCGCTC TCGTTCGCCT GGAGTATTTC ACGCAGCATC AGGCCTAACC TCAGACGTTG


       1330        1340        1350        1360        1370        1380
TCGACTCCAT GAAGTCGGAA TCGCTAGTAA TCGTGGATCA GAATGCCACG GTGAATACGT
AGCTGAGGTA CTTCAGCCTT AGCGATCATT AGCACCTAGT CTTACGGTGC CACTTATGCA
                                                       GC CACTTATGCA
```

1743

```
       1390        1400        1410        1420        1430        1440
TCCCGGGCCT TGTACACACC GCCCGTCACA CCATGGGAGT GGGTTGCAAA AGAAGTAGGT
AGGGCCCGGA ACATGTGTGG CGGGCAGTGT GGTACCCTCA CCCAACGTTT TCTTCATCCA
AGGGCCCGGA ACATG
‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾‾
```

1743

```
       1450        1460        1470        1480        1490        1500
AGCTTAACCT TCGGGAGGGC GCTTACCACT TTGTGATTCA TGACTGGGGT GAAGTCGTAA
TCGAATTGGA AGCCCTCCCG CGAATGGTGA AACACTAAGT ACTGACCCCA CTTCAGCATT


       1510        1520        1530        1540        1550
CAAGGTAACC GTAGGGGAAC CTGCGGTTGG ATCACCTCCT TA........
GTTCCATTGG CATCCCCTTG GACGCCAACC TAGTGGAGGA AT........
```

FIGURE 89

Sheet 1/3

```
                                        AGAGTTTGATCCTGGCTCAG
1638 (SEQ ID NO:151)                                                                                        GGCGGACGGGG
E.colirrsE (SEQ ID NO:158)0  ...AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCGCAGGCCTAACACATGCA
Cam.jejun5 (SEQ ID NO:159)0  ~TTTTATGGAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCGTGCCTAATACATGCA
Stp.aureus (SEQ ID NO:160)0  ..TTTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA

ER10 (SEQ ID NO:152)
E.colirrsE   60 AGTCGAACGGTAACAG-----GAAGAAGCTTGCTTCTTT----GCTGACGAGTGGCGACGGG
Cam.jejun5   62 AGTCGAACGAT------GAAGCTTCTAGCTTGCTAGAAGTGGA-----TTAGTGGCGCACGGG
Stp.aureus   61 AGTCGAGCGAA------CGGACGAGAAGCTTGCTTCTCTGATG---TT-AGCGGCGGACGGGG

ER10         TGAGTAA
E.colirrsE  114 TGAGTAATGTCTGGGA-AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA
Cam.jejun5  114 TGAGTAAGGTATAGTTAATCTGCCCTACACAAGAGAGACAACAGTTGGAAACGACTGCTAATA
Stp.aureus  113 TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATA

E.colirrsE  175 CCGCATAAC------GTCGCAAGAC------CAAAGAGGGGGACCTTCG-GGCCTCTTG
Cam.jejun5  176 CTCTATACTCCTGCTTAACACAAGTTGAGTAGG-GAAAG------TTTTT------CG
Stp.aureus  175 CCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGT----CTT----GCTGTCA

E.colirrsE  221 CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGA
Cam.jejun5  221 GTGTAGGATGAGACTATATAGTATCAGCTAGTTGGTAAGGTAATGCTTACCAAGGCTATGA
Stp.aureus  229 CTTATAGATGATCCGCGCTGCATTAGCTAGTTGGTGGTAACGGCTTACCAAGGCAACGA

E.colirrsE  283 TCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
Cam.jejun5  283 CGCTTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA
Stp.aureus  291 TACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA  **ACTCCTA**

E.colirrsE  345 CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
Cam.jejun5  345 CGGGAGGCAGCAGTAGGGAATATTGCGCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTG
Stp.aureus  353 CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTG  **CGGGAGGCAGCAG**

E.colirrsE  407 TATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA-GGGAGTAAAGTTAAT
Cam.jejun5  407 GAGGATGACACTTTTCGGAGCGTAAACTCCTTTTCTTAGGGAAG-------------AATT
Stp.aureus  415 AGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAAC

E.colirrsE  468 ACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Cam.jejun5  455 C-------TGACGGTACCTAAGGAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Stp.aureus  476 _TGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG
```

FIGURE 89

```
E.colirrsE   530  GTAATACGGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTT
Cam.jejun5   506  GTAATACGGAGGGTGCAAGCGTTACTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGATT
Stp.aureus   538  GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT

E.colirrsE   592  GTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTT
Cam.jejun5   568  ATCAAGTCTCTTGTGAAATCTAATGGCTTAACCATTAAACTGCTTGGGAAACTGATAGTCTA
Stp.aureus   600  TTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT

E.colirrsE   654  GAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
Cam.jejun5   630  GAGTGAGGGAGGGCAGATGAATGGAATTGGTGGTGTAGGGGTAAAATCCGTAGATATCACCAAGA
Stp.aureus   662  GAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGA

E.colirrsE   716  ATACCGGTGGCGAAGGCGGCCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
Cam.jejun5   692  ATACCCATTGCGAAGGCGATCTGCTGGAACTCAACTGACGCTAAGGCGCGAAAGCGTGGGGA
Stp.aureus   724  ACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGA

E.colirrsE   778  GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGC
Cam.jejun5   754  GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTACACTAGTTGTTGGGGT
Stp.aureus   786  TCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGG

E.colirrsE   840  C-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGC
Cam.jejun5   816  G-CTAGT-CATCTCAGTAATGCGATCAGCTAACGCATTAAGTACCGCCTGGGGAGTACGGTCGC
Stp.aureus   848  GT-TTCCGCCCCTTAGTGCTGCGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGC

E.colirrsE   900  AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Cam.jejun5   876  AAGGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Stp.aureus   909  AAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT

E.colirrsE   962  CGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAAT
Cam.jejun5   938  CGAAGATACGCGAAGAACCTTACCTGGGCTTGATATCCTAAGAATCCTTAGAGATAAGAGG
Stp.aureus   971  CGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCC

E.colirrsE   1024 GTG--CCTTCGGG--AA-CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
Cam.jejun5   1000 GTGCTAGCTTGCTAGAA-CTTAGAGACAGGTGCTGCACGGCTGTCGTCAGCTCGTGTCGTGA
Stp.aureus   1033 TTCC-CCTTCGGG--GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA

                   GCAACGAGCGCAACCC
SB-1
E.colirrsE   1081 AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGG-CC
Cam.jejun5   1061 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTATTTAGTTGCTAACGGTTCGG-CC
Stp.aureus   1092 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCA_TTAAGT_T
```

FIGURE 89

Sheet 3/3

```
SB-3   (SEQ ID NO:157)                                                  ATGACGTCAAGTCATC
SB-4   (SEQ ID NO:154)                                                  ATGACGTCAAGTCATC
E.colirrsE 1142 GGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGATGACGTCAAGTCATC
Cam.jejun5 1122 GAGCACTCTAAATAGACTGCCTTCG_TAAGGAGGAGGAAGGTGTGACGACGTCAAGTCATC
Stp.aureus 1152 GGGCACTCTAAGTTGACTGCCGGTGACAAACCGAGGAAGGTGGGATGACGTCAAATCATC


SB-3   ATGGCCCTTA
SB-4   ATGGCCCTTACGA
E.colirrsE 1204 ATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCCATACAAAGAGAAGCGACCTC
Cam.jejun5 1183 ATGGCCCTTATGCCCAGGGCGACACACGTGCTACAATGGCATATAGAATGAGACGCAATACC
Stp.aureus 1214 ATGCCCCTTATGATTTGGGCTACACACGTGCTACAACACAATACAAAGGGCAGCGAAACC


E.colirrsE 1266 GCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
Cam.jejun5 1245 GCGAGGTGGAG_CAAATCTATAAAATATGTCCCAGTTCGGATTGTTCTCTGCAACTCGAGAG
Stp.aureus 1276 GCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTTCTCAACTCGACTA


E.colirrsE 1328 CATGAAGTCGGAATCGCTAGTAATCGTGGATCAGA_ATGCCACGGTGAATACGTTCCCGGGC
Cam.jejun5 1306 CATGAAGCCGGAATCGCTAGTAATCGTAGATCAGCCATGCTACGGTGAATACGTTCCCGGGT
Stp.aureus 1338 CATGAAGCTGGAATCGCTAGTAATCGTAGATCAGC_ATGCTACGGTGAATACGTTCCCGGGT
1743(compl)                                       CGGTGAATACGTTCCCGGGC


E.colirrsE 1389 CTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAGAAGTAGGTAGCTTAACCT
Cam.jejun5 1368 CTTGTACTCACCGCCCGTCACACCATGGGAGTTGATTCACTCGAAGCCGGAATACT_-A_A
Stp.aureus 1399 ATTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCT
1743(compl) CTTGTAC


E.colirrsE 1451 TCG_GGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCG
Cam.jejun5 1427 AC--T_AGTTACCGTCCACAGTGGAATCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCG
Stp.aureus 1461 TTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGTGAAGTCGTAACAAGGTAGCCG


E.colirrsE 1512 TAGGGGAACCTGCGGTTGGATCACCTCCTTA---
Cam.jejun5 1485 TAGGAGAACCTGCGGTTGGATCACCTCCT-----
Stp.aureus 1523 TATCGGAAGGTGCGGCTGGATCACCTCCTTTCT-
```
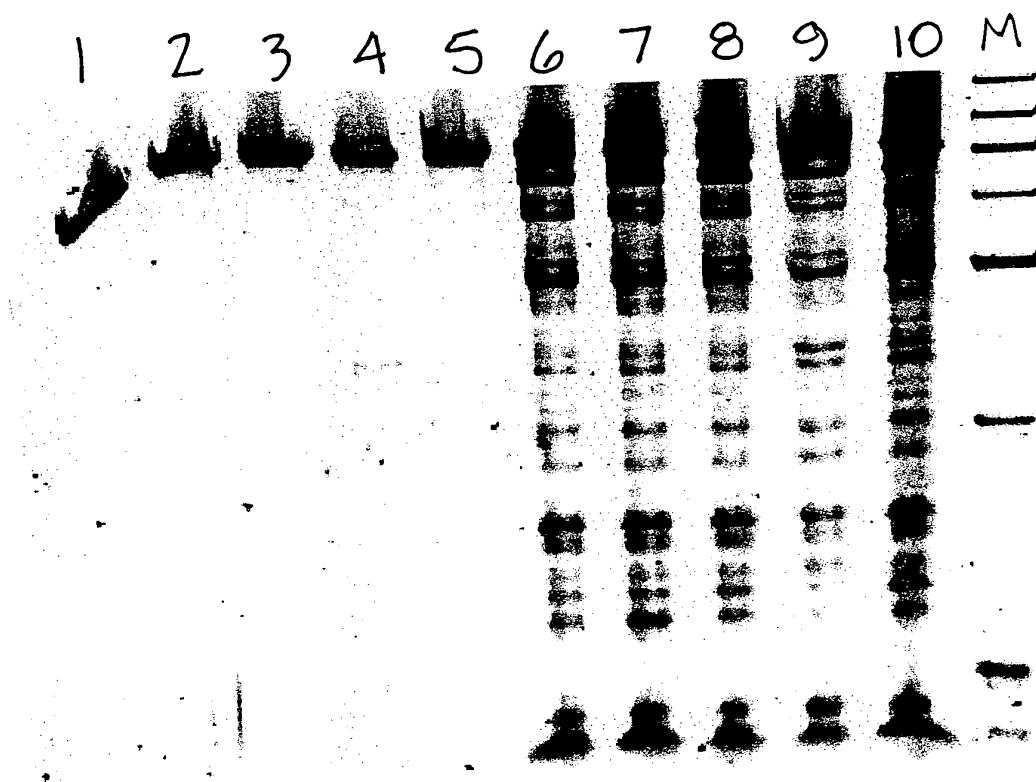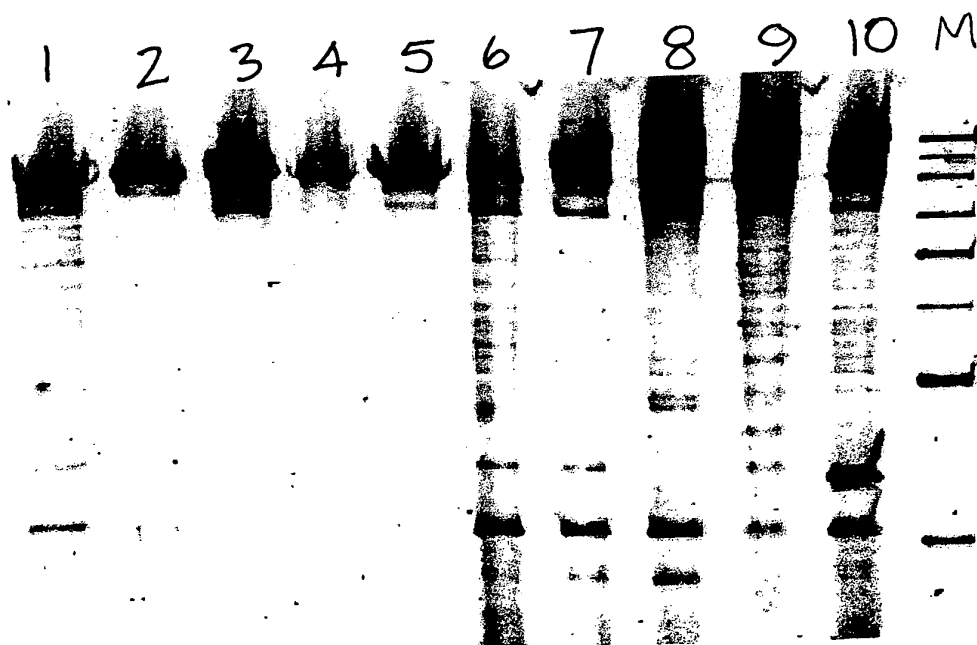
FIGURE 90

FIGURE 91

A.

B.

FIGURE 92
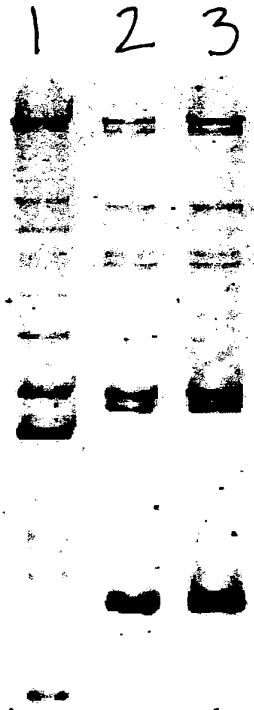
FIGURE 93

FIGURE 94